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(57) Abstract

The present invention provides novel complementary DNA (cDNA) sequences encoding human matrix metalloprotease 19 proteins. The present invention also provides recombinant DNA molecules encoding matrix metalloprotease 19 polypeptides and processes for producing the novel proteins. The cDNA is cloned into expression vectors for expression in recombinant hosts. The cDNA is useful to produce recombinant full length MMP19s or fragments thereof. The cDNA and the recombinant proteins derived therefrom and/or antibodies to the proteins are useful in diagnostic assays and for the development of therapeutic agents that affect MMP function.

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HUMAN MATRIX METALLOPROTEASE GENE, PROTEINS ENCODED THEREFROM AND METHODS OF USING SAME

Background of the Invention

1. Field of the Invention

The present invention relates generally to therapeutic and diagnostic agents in cancer and inflammatory diseases. More specifically, the present invention relates to polynucleotide sequences encoding matrix metalloprotease proteins, as well as methods which utilize these sequences, which are useful for the detection, diagnosis, staging, monitoring, prognosis, prevention, or treatment of cancer or inflammatory diseases.

2. Description of the Related Art

The matrix metalloproteases (MMPs) are a multi-enzyme family capable of completely degrading the components of the extracellular matrix (ECM), their natural substrates (W.B. Ennis, and L. M. Matrisian, *J. Neuro-Oncology*, **18:** 105-109 (1994)). The ECM is a meshwork of cells and various types of collagens and proteoglycans, collectively called connective tissue, which provides mechanical support and helps to maintain the structural integrity of tissues and organs. The function of the ECM is particularly apparent in articular cartilage where it provides cushioning and ease of movement between bones in joints. The MMPs are secreted by the cellular components of the ECM (fibroblasts, chondrocytes and synoviocytes) and inflammatory cells (neutrophils and macrophages) in inactive forms (zymogens) which are converted extracellularly to the active enzymes by various proteinases. Normally MMPs function in a highly regulated fashion as part of the physiological turnover of the ECM, effectively renewing and remodeling the ECM. However, in the clinical features of several diseases, the ECM is degraded and there is much evidence to support that MMPs play a significant pathological role in ECM degradation.

At least fourteen members of the MMP family have been identified and most assigned EC numbers; several more have been discovered recently. They can be classified generally according to four subgroups based on substrate preference or cellular localization; *e.g.*, collagenases prefer Type I and II collagen, gelatinases prefer Type IV collagen, and stromelysins prefer proteoglycans (L. M. Matrisian, *Bio. Essays* **14**: 455-463 (1992)). The fourth subgroup are the membrane type MMPs (mtMMPs) which are characterized by the presence of a hydrophobic transmembrane domain near the C-terminus for anchoring the protein in the cell membrane. All of the other MMPs are secreted into the extracellular milieu.

Most of the known MMPs contain zinc in their catalytic sites and require calcium for activity. The major human MMPs have been cloned and exhibit greater than 50% homology. They contain a leader sequence for signaling their secretion by cells; a highly conserved pro-enzyme sequence removed upon activation; a catalytic site with a highly conserved zinc

binding domain; and a carboxy terminal region containing a conserved sequence similar to hemopexin, a heme binding protein. Although MMPs can be readily activated *in vitro* using mercurial compounds or trypsin, the precise mechanism for propeptide removal and activation of MMPs *in vivo* is not understood. Some MMPs can undergo an autoactivation process, while recent evidence indicates that membrane type MMPs may function as activators of other MMPs.

A growing line of evidence implicates the MMPs as important enzymes in cancer metastasis. Although different cancer cell lines have been shown to express various MMPs when grown in culture, gelatinase A in particular has been the focus of a number of recent studies which demonstrates its role in the invasiveness of cancer cells (W. G. Stetler-Stevenson, *et al.*, *FASEB J.* **7**: 1434-1441 (1993)). For example, gelatinase A is found in the urine of bladder cancer patients and specific monoclonal antibodies have been used to detect the enzyme in breast tumor sections (I.M. Margulies, *et al.*, **1**: 467-474 (1992)). The enzyme is expressed in an invasive prostate cancer cell line (PC-3 ML) and cells transfected with the gelatinase A gene are capable of extravasation when injected into mice (M. E. Stearns, and M. Wang, *Oncology Res.* **6**: 195-201 (1994)). These studies and others implicate the involvement of gelatinase A in tumor metastasis, and suggest that inhibitors of this enzyme may offer therapeutic potential in certain forms of cancer. A broad spectrum matrix metalloproteinase inhibitor has been shown to decrease the tumor burden of mice bearing ovarian carcinoma xenographs (B. Davies, *et al.*, *Cancer Res.* **53**: 2087-2091 (1993)). This compound (BB-94, batimastat) is currently being evaluated in clinical trials for malignant ascites (S.A. Watson, *et al.*, *Cancer Research*, **55**: 3629-3633 (1995)); however, its poor bioavailability necessitates parental administration. Gelatinase A selective succinyl hydroxamates have been suggested as anti-cancer agents as well, yet these compounds possess the same peptidic backbone as batimastat (Porter, J. R.; Beeley, N. R. A.; Boyce, B. A.; Mason, B.; Millican, A.; Millar, K.; Leonard, J.; Morphy, J. R.; O'Connell, J. P. Potent and selective inhibitors of gelatinase A 1. Hydroxamic acid derivatives. *Bioorg. Med. Chem. Lett.*, **4**: 2741-2746 (1994)). More recently, an orally active, broad spectrum, MMP inhibitor (BB-2516, marimastat) was reported to stop progression of colorectal, ovarian, prostatic and pancreatic cancer (R. P. Beckett, *et al.*, *D. D. T.*, **1**: 16-26 (1996)).

Several lines of evidence indicate that the unregulated activity of MMPs is responsible for the joint degradation observed in rheumatoid arthritis and osteoarthritis. In these human arthritides, activated forms of the MMPs and their products (glycosaminoglycans and collagen fragments) are found in synovial fluids and joint tissues in abnormally high amounts (see E.D. Harris Jr., Role of collagenase in joint destruction. *The Joints and Synovial Fluid*, Vol., 1, Sokologg, L., Ed., Orlando, FL, Academic Press, 1977, T.E. Cawston, *et al.*, *Arthritis Rheum.*, **27**: 285-290 (1984), Cawston, T. *Ann. Rheumatic Diseases.*, **52**: 769-770 (1993),

Y.L. Okada, *et al.*, *J. Biol. Chem.*, **261**: 14245-14255 (1986), D.L. Scott, *et al.*, *Molec. Aspects Med.*, **12**: 341-394 (1991), and E.D. Harris, *et al.*, *Arthritis Rheum.*, **12**: 92-102 (1992)). These arthritic tissues also show a greater-than-normal expression of MMPs (S.S. McCachren, *Arthritis and Rheumatism* , **34**: 1085-1093 (1991), A.J.P. Docherty and G. Murphy, *Ann. Rheumat. Dis.*, **49**: 469-479 (1990)) which is induced by cytokines and growth factors, also found abundantly in these tissues (D.L. Scott, *et al.*, (1991), *supra*, S.M. Frisch and H.E. Ruley, *J. Biol. Chem.*, **262**: 16300-16304 (1987)). In addition, the activities of MMPs in normal tissues are thought to be regulated by the presence of endogenous tissue inhibitors of MMPs, (TIMPs, see T.E. Cawston, *Curr. Med. Lit. Rheum.*, **3**: 127-0 (1984)). The ratio of the amounts of TIMP and MMPs is thought to maintain a balance between the rates of degradation and synthesis of ECM. In tissues from rheumatoid arthritics, an abnormally high expression of MMPs results in an imbalance of these enzymes and degradation of ECM (S.S. McCachren (1991) and T.E. Cawston (1984), *supra*). Thus in arthritis, inhibition of the exacerbated degradative activities of MMPs by specific agents could help restore this balance. In rodent models which mimic the biochemical features of arthritis, there is evidence that the combined action of proteoglycan loss (due to stromelysin activity) and cartilage degradation (due to collagenase) are early events in this disease (R.M. Hembry, *et al.*, *Am. J. Pathol.*, **143**: 628-642 (1993), K.A. Hasty, *et al.*, *Arthritis and Rheumatism* , **33**: 388-397 (1990)). Several prototype inhibitors of MMPs have been shown to reduce cartilage degradation in these animal models (M.J. DiMartino, *et al.*, *J. Cell Biochem.* suppl., **19E**: 179 (1991), P. Brown, *et al.*, Orally active inhibitors of cartilage degradation. Abst. # 81, Abstracts of Inflammation '93, Vienna, Austria, p. 29. (1993)).

There is much evidence to suggest that MMPs mediate the migration of inflammatory cells into endothelium (D., Moscatelli and D.B. Rifkin, *Biochim. Biophys. Acta.*, **948**: 67-85 (1988), P. Zaoui, *et al.*, Matrix metalloproteases (MMP) exocytosis from neutrophils is inhibited by endothelial adhesion. Abst. # 83, Abstracts of Inflammation '93, Vienna, Austria, p. 29 (1993)) participating in periodontal diseases (H. Birkedal-Hansen, *J. Peridontol.*, **64**: 474-484 (1993)) and facilitating the growth of atherosclerotic plaques (A. M. Henny, *et al.*, *Proc. Natl. Acad. Sci.*, **8**: 8154-8158 (1991)). Recently, gelatinase-A was reported to promote cleavage of the amyloid protein precursor which would suggest a role in Alzheimers disease for this MMP (N. Peress, *et al.*, *J. Neuropathol. Exp. Neurol.*, **54**: 16-22 (1995), R.N. Lipage, *et al.*, *FEBS Lett.*, **377**: 267-270 (1995)). Thus, there is compelling evidence that MMPs play an important role in arthritis and other inflammatory diseases and that targeted inhibition of these proteinases by pharmaceutical agents could have beneficial effects.

It would be advantageous to provide specific methods and reagents for the diagnosis, staging, prognosis, monitoring, prevention or treatment of diseases and conditions associated with imbalances in the production or activity of MMPs or to indicate possible predisposition to

these conditions. Such methods would include assaying a test sample for products of the gene. Such methods would comprise making cDNA from mRNA in the test sample, amplifying (when necessary) portions of the cDNA corresponding to the gene or a fragment thereof, and detecting the cDNA product as an indication of the presence of the cancer; or detecting translation products of the mRNAs comprising gene sequences as an indication of the presence of the disease. These reagents include polynucleotide(s), or fragment(s) thereof which may be used in diagnostic methods such as reverse transcriptase-polymerase chain reaction (RT-PCR), PCR, or hybridization assays of biopsied tissue; or proteins which are the translation products of such mRNAs; or antibodies directed against these proteins. Such assays would include methods for assaying a sample for product(s) of the gene and detecting the product(s) as an indication of disease. Drug treatment or gene therapy for conditions or diseases associated with these detected diseases and conditions then can be based on these identified gene sequences or their expressed proteins, and efficacy of any particular therapy can be monitored using the diagnostic methods disclosed herein.

Studies to understand the role of MMPs in tumor growth, metastases, or inflammatory conditions and diseases such as arthritis and their potential as a therapeutic or diagnostic tools are limited to previously described MMP proteins. It would be advantageous to identify novel human MMPs which may be directly involved in the physiology of, for example, tumor growth, extravasation, or invasion. Isolation of DNA sequences encoding human MMPs would permit more extensive studies on the association and regulation of individual MMPs in specific cancers or tumor types, and in diseases such as arthritis or other inflammatory conditions. In addition, the identification of tissue-specific or disease-specific MMPs would provide more direct targets for therapeutics designed to attenuate these diseases.

Summary of the Invention

This present invention provides an isolated and purified polynucleotide encoding a matrix metalloprotease (provisionally named MMP19), polynucleotide fragments thereof, expression vectors containing those polynucleotides, host cells transformed with those expression vectors, processes for making the MMP protein using those polynucleotides and vectors, isolated and purified MMP protein and polypeptide fragments thereof, and antibodies raised to synthetic peptides derived from the MMP protein. The invention also provides diagnostic assays to identify the presence of the MMP polynucleotide or polypeptide, assays used to identify agents that affect the function of the MMP polynucleotide or polypeptide, and the use as therapeutic agents of the MMP polynucleotide, polypeptides, or antibodies.

The cDNA clone was obtained by screening a human cDNA expression database with a consensus sequence to twelve other human MMPs. The sequence of the partial cDNA isolated

indicated that the gene product is a novel MMP protein that is expressed in a limited number of tissues.

Brief Description of the Drawings

FIG. 1 shows the MMP consensus sequence used to query the LifeSeq™ database. The functional motifs present in all MMPs are double underlined.

FIG. 2 shows the complete nucleotide and translated sequence (frame 3) of EST #907334 in LifeSeq™. A putative cysteine switch motif (PRCGVTD which is SEQ ID NO:8) and a putative furin recognition site (RKKR which is SEQ ID NO:9) are double underlined.

FIG. 3 shows the length and position of individual sequencing reactions to generate the double stranded nucleotide sequence of the insert in clone #907334. The boxes at the bottom are graphic representations of the three forward reading frames with start codons shown as short upward lines and stop codons as longer downward lines. Frame 1 contains the longest open reading frame.

FIG. 4 shows the doubled stranded complete nucleotide sequence (SEQ ID NO:1 and SEQ ID NO:2, top and bottom strand respectively) of the MMP19 gene and the translated amino acid sequence (SEQ ID NO:9) of MMP19 (shown beneath in single letter codes). Indicated in bold type are the structural domains found in other MMPs. The cysteine switch, furin recognition site, and zinc binding site are double underlined. The putative poly adenylation signal in the 3' untranslated region of the mRNA is single underlined. Boxes indicate the position of the 3' end of the primers used in the synthesis of DNA constructs for the expression of the catalytic portion of MMP19.

FIG. 5 shows the alignment of the polypeptide encoded by SEQ ID NO:10 with the amino acid sequences of all known human MMPs, namely, matrilysin (GenBank Accession No. L22524, SEQ ID NO:18), Gelatinase A (GenBank Accession No. M55593, SEQ ID NO:19), Gelatinase B (GenBank Accession No. J05070, SEQ ID NO:20), Stromelysin 1 (GenBank Accession No. J03209, SEQ ID NO:21), Stromelysin 2 (GenBank Accession No. X07820, SEQ ID NO:22), Stromelysin 3, (GenBank Accession No. X57766, SEQ ID NO:23), Collagenase 1 (GenBank Accession No. M13509, SEQ ID NO:24), Collagenase 2 (GenBank Accession No. J05556, SEQ ID NO:25), Collagenase 3 (GenBank Accession No. X75308, SEQ ID NO:26), MMP12 (GenBank Accession No. L23808, SEQ ID NO:27), MMP18 (GenBank Accession No. Y08622 or X92521, SEQ ID NO:28), mt1MMP (GenBank Accession No. D26512, SEQ ID NO:29), mt2MMP (GenBank Accession No. Z48482, SEQ

ID NO:30), mt3MMP (GenBank Accession No. D50477, SEQ ID NO:31), and mt4MMP (GenBank Accession No. X89576, SEQ ID NO:32). The consensus sequence was generated using a plurality of 5. Characteristic motifs found in MMPs are double underlined.

FIG. 6 shows the alignment and tissue distibution of ESTs found in LifeSeq™ which are 100% identical to regions of MMP19 cDNA.

FIG. 7 shows a PhosphoImage of a Northern blot of human RNA (2μg of poly A^+ RNA/lane) probed with a ^{32}P-labeled MMP19 cDNA probe.

Detailed Description of the Invention

The present invention provides isolated and purified polynucleotides that encode a human matrix metalloprotease, fragments thereof, expression vectors containing those polynucleotides, host cells transformed with those expression vectors, a process of making the MMP using those polynucleotides and vectors, and isolated and purified recombinant MMP and polypeptide fragments thereof.

The present invention also provides for the use of MMP19 in developing treatments for any disorder mediated (directly or indirectly) by insufficient amounts or production of MMP19 protein. Purified human MMP19 protein may be administered to a patient with such a condition. Alternatively, gene therapy techniques for producing MMP19 polypeptide *in vivo* are also provided.

The present invention also provides methods for assaying a test sample for products of the MMP gene, which comprises making cDNA from mRNA in the test sample, and detecting the cDNA as an indication of the presence of the MMP gene. The method may include an amplification step, wherein portions of the cDNA corresponding to the gene or fragment thereof is amplified. Methods also are provided for assaying for the translation products of mRNAs. Test samples which may be assayed by the methods provided herein include tissues, cells, body fluids and secretions. The present invention also provides reagents such as oligonucleotide primers and polypeptides which are useful in performing these methods.

Portions of the nucleic acid sequences disclosed herein are useful as primers for the reverse transcription of RNA or for the amplification of cDNA; or as probes to determine the presence of certain cDNA sequences in test samples. Also disclosed are nucleic acid sequences which permit the production of encoded polypeptide sequences which are useful as standards or reagents in diagnostic immunoassays, targets for pharmaceutical screening assays and/or as components or target sites for various therapies. Monoclonal and polyclonal antibodies directed against at least one epitope contained within these polypeptide sequences are useful for diagnostic tests as well as delivery agents for therapeutic agents and for

screening for diseases or conditions associated with cancers, arthritis, or inflammation. Isolation of sequences of other portions of the gene of interest can be accomplished by utilizing probes or PCR primers derived from these nucleic acid sequences, thus allowing additional probes and polypeptides of the genome of interest to be established, which also will be useful in the diagnosis, prognosis and/or treatment of diseases and conditions characterized by the MMP gene disclosed herein.

The techniques for determining the amino acid sequence "similarity" are well-known in the art. In general, "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences. The techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded therein, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more polynucleotide sequences can be compared by determining their "percent identity." Two amino acid sequences likewise can be compared by determining their "percent identity." The programs available in the Wisconsin Sequence Analysis Package (available from Genetics Computer Group, Madison, WI), for example, the GAP program, are capable of calculating both the identity between two polynucleotides and the identity and similarity between two polypeptide sequences, respectively. Other programs for calculating identity or similarity between sequences are known in the art.

The compositions and methods described herein will enable the identification of certain markers as indicative of cancer, arthritis, or inflammation resulting from or associated with an MMP19 disorder; the information obtained therefrom will aid in the diagnosis, staging, monitoring, prognosis and/or therapy of those same diseases or conditions. Test methods include, for example, probe assays which utilize the sequence(s) provided herein and which also may utilize nucleic acid amplification methods such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR); and hybridization. In addition, the nucleotide sequences provided herein contain open reading frames from which one or more immunogenic epitopes may be found. Such epitopes are believed to be unique to the disease state or condition associated with MMP19 related cancer, arthritis, or inflammation. The uniqueness of any epitope may be determined by its immunological reactivity with the specific MMP gene in diseased tissues and lack of immunological reactivity with non-diseased tissues. Methods for determining immunological reactivity are well-known and include but are not limited to, for example, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA),

hemagglutination (HA), fluorescence polarization immunoassay (FPIA); chemiluminescent immunoassay (CLIA), and others; several examples of suitable methods are described herein.

Unless otherwise stated, the following terms shall have the following meanings:

A polynucleotide "derived from" a designated sequence refers to a polynucleotide sequence which is comprised of a sequence of approximately at least about 6 nucleotides, is preferably at least about 8 nucleotides, is more preferably at least about 10-12 nucleotides, and even more preferably is at least about 15-20 nucleotides corresponding, i.e., identical to or complementary to, a region of the designated nucleotide sequence. The sequence may be complementary to or identical to a sequence which is unique to a particular polynucleotide sequence as determined by techniques known in the art. Comparisions to sequences in databanks, for example, can be used as a method to determine the uniqueness of a designated sequence. Regions from which sequences may be derived include but are not limited to regions encoding specific epitopes, as well as non-translated and/or non-transcribed regions.

The derived polynucleotide will not necessarily be derived physically from the nucleotide sequence of interest under study, but may be generated in any manner, including but not limited to chemical synthesis, replication, reverse transcription or transcription, which is based on the information provided by the sequence of bases in the region(s) from which the polynucleotide is derived; as such, it may represent either a sense or an antisense orientation of the original polynucleotide. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with an intended use.

The term "primer" denotes a specific oligonucleotide sequence complementary to a target nucleotide sequence and used to hybridize to the target nucleotide sequence and serve as an initiation point for nucleotide polymerization catalyzed by either DNA polymerase, RNA polymerase or reverse transcriptase.

The term "probe" denotes a defined nucleic acid segment (or nucleotide analog segment, i.e., PNA) which can be used to identify specific DNA or RNA present in samples bearing the complementary sequence.

A "polypeptide" or "amino acid" sequence derived from a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence or a portion thereof wherein the portion consists of at least 3 to 5 contiguous amino acids, and more preferably at least 8 to 10 contiguous amino acids, and even more preferably 15 to 20 continguous amino acids, or which is immunologically identifiable with a polypeptide encoded in the sequence.

A "recombinant polypeptide" as used herein means at least a polypeptide which by virtue of its origin or manipulation is not associated with all or a portion of the polypeptide with which it is associated in nature and/or is linked to a polypeptide other than that to which it

is linked in nature. A recombinant or derived polypeptide is not necessarily translated from a designated nucleic acid sequence. It also may be generated in any manner, including chemical synthesis or expression of a recombinant expression system.

The term "synthetic peptide" as used herein means a polymeric form of amino acids of any length, which may be chemically synthesized by methods well-known to the routineer. These synthetic peptides are useful in various applications.

The term "polynucleotide" as used herein means a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, the term includes double- and single-stranded DNA, as well as, double- and single-stranded RNA. It also includes modifications, such as methylation or capping, and unmodified forms of the polynucleotide.

"A sequence corresponding to a cDNA" means that the sequence contains a polynucleotide sequence that is identical to or complementary to a sequence in the designated DNA. The degree (or "percent") of identity or complementarity to the cDNA will be approximately 50% or greater, will preferably be at least about 70% or greater, and more preferably will be at least about 90%. The sequence that corresponds will be at least about 50 nucleotides in length, will preferably be about 60 nucleotides in length, and more preferably, will be at least about 70 nucleotides in length. The correspondence between the gene or gene fragment of interest and the cDNA can be determined by methods known in the art, and include, for example, a direct comparison of the sequenced material with the cDNAs described, or hybridization and digestion with single strand nucleases, followed by size determination of the digested fragments.

"Purified polynucleotide" refers to a polynucleotide of interest or fragment thereof which is essentially free, i.e., contains less than about 50%, preferably less than about 70%, and more preferably, less than about 90% of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density. Thus, "purified polypeptide" means a polypeptide of interest or fragment thereof which is essentially free, that is, contains less than about 50%, preferably less than about 70%, and more preferably, less than about 90% of cellular components with which the polypeptide of interest is naturally associated. Methods for purifying are known in the art.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, which is separated from some or all of the coexisting

materials in the natural system, is isolated. Such polynucleotide could be part of a vector and/or such polynucleotide or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural environment.

"Polypeptide" as used herein indicates a molecular chain of amino acids and does not refer to a specific length of the product. Thus, peptides, oligopeptides and proteins are included within the definition of polypeptide. This term, however, is not intended to refer to post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like.

"Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vector or other transferred DNA, and include the original progeny of the original cell which has been transfected.

As used herein "replicon" means any genetic element, such as a plasmid, a chromosome or a virus, that behaves as an autonomous unit of polynucleotide replication within a cell.

A "vector" is a replicon in which another polynucleotide segment is attached, such as to bring about the replication and/or expression of the attached segment.

The term "control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include promoter, ribosomal binding site and terminators; in eukaryotes, such control sequences generally include promoters, terminators and, in some instances, enhancers. The term "control sequence" thus is intended to include at a minimum all components whose presence is necessary for expression, and also may include additional components whose presence is advantageous, for example, leader sequences.

"Operably linked" refers to a situation wherein the components described are in a relationship permitting them to function in their intended manner. Thus, for example, a control sequence "operably linked" to a coding sequence is ligated in such a manner that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "open reading frame" or "ORF" refers to a region of a polynucleotide sequence which encodes a polypeptide; this region may represent a portion of a coding sequence or a total coding sequence.

A "coding sequence" is a polynucleotide sequence which is transcribed into mRNA and translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon

at the 5' -terminus and a translation stop codon at the 3' -terminus. A coding sequence can include, but is not limited to, mRNA, cDNA, and recombinant polynucleotide sequences.

The term "immunologically identifiable with/as" refers to the presence of epitope(s) and polypeptide(s) which also are present in and are unique to the designated polypeptide(s). Immunological identity may be determined by antibody binding and/or competition in binding. These techniques are known to the routineer and also are described herein. The uniqueness of an epitope also can be determined by computer searches of known data banks, such as GenBank, for the polynucleotide sequences which encode the epitope, and by amino acid sequence comparisons with other known proteins.

As used herein, "epitope" means an antigenic determinant of a polypeptide. Conceivably, an epitope can comprise three amino acids in a spatial conformation which is unique to the epitope. Generally, an epitope consists of at least five such amino acids, and more usually, it consists of at least eight to ten amino acids. Methods of examining spatial conformation are known in the art and include, for example, x-ray crystallography and two-dimensional nuclear magnetic resonance.

A "conformational epitope" is an epitope that is comprised of specific juxtaposition of amino acids in an immunologically recognizable structure, such amino acids being present on the same polypeptide in a contiguous or non-contiguous order or present on different polypeptides.

A polypeptide is "immunologically reactive" with an antibody when it binds to an antibody due to antibody recognition of a specific epitope contained within the polypeptide. Immunological reactivity may be determined by antibody binding, more particularly by the kinetics of antibody binding, and/or by competition in binding using as competitor(s) a known polypeptide(s) containing an epitope against which the antibody is directed. The methods for determining whether a polypeptide is immunologically reactive with an antibody are known in the art.

As used herein, the term "immunogenic polypeptide containing an epitope of interest" means naturally occurring polypeptides of interest or fragments thereof, as well as polypeptides prepared by other means, for example, by chemical synthesis or the expression of the polypeptide in a recombinant organism.

The term "transformation" refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion. For example, direct uptake, transduction or f-mating are included. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host genome.

"Treatment" refers to prophylaxis and/or therapy.

The term "individual" as used herein refers to vertebrates, particularly members of the mammalian species and includes but is not limited to domestic animals, sports animals, primates and humans; more particularly the term refers to humans.

The term "sense strand" or "plus strand" (or "+") as used herein denotes a nucleic acid that contains the sequence that encodes the polypeptide. The term "antisense strand" or "minus strand" (or "-") denotes a nucleic acid that contains a sequence that is complementary to that of the "plus" strand.

The term "test sample" refers to a component of an individual's body which is the source of the analyte (such as, antibodies of interest or antigens of interest). These components are well known in the art. These test samples include biological samples which can be tested by the methods of the present invention described herein and include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, urine, lymph fluids, and various external secretions of the respiratory, intestinal and genitorurinary tracts, tears, saliva, milk, white blood cells, myelomas and the like; biological fluids such as cell culture supernatants; fixed tissue specimens; and fixed cell specimens.

"Purified product" refers to a preparation of the product which has been isolated from the cellular constituents with which the product is normally associated, and from other types of cells which may be present in the sample of interest.

"PNA" denotes a "peptide nucleic acid analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. "MA" denotes a "morpholino analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. See, for example, U.S. Patent No. 5,378,841, which is incorporated herein by reference. PNAs are neutrally charged moieties which can be directed against RNA targets or DNA. PNA probes used in assays in place of, for example, the DNA probes of the present invention, offer advantages not achievable when DNA probes are used. These advantages include manufacturability, large scale labeling, reproducibility, stability, insensitivity to changes in ionic strength and resistance to enzymatic degradation which is present in methods utilizing DNA or RNA. These PNAs can be labeled with such signal generating compounds as fluorescein, radionucleotides, chemiluminescent compounds, and the like. PNAs or other nucleic acid analogs such as MAs thus can be used in assay methods in place of DNA or RNA. Although assays are described herein utilizing DNA probes, it is within the scope of the routineer that PNAs or MAs can be substituted for RNA or DNA with appropriate changes if and as needed in assay reagents.

"Analyte," as used herein, is the substance to be detected which may be present in the test sample. The analyte can be any substance for which there exists a naturally occurring specific binding member (such as, an antibody), or for which a specific binding member can be prepared. Thus, an analyte is a substance that can bind to one or more specific binding

members in an assay. "Analyte" also includes any antigenic substances, haptens, antibodies, and combinations thereof. As a member of a specific binding pair, the analyte can be detected by means of naturally occurring specific binding partners (pairs) such as the use of intrinsic factor protein as a member of a specific binding pair for the determination of Vitamin B12, the use of folate-binding protein to determine folic acid, or the use of a lectin as a member of a specific binding pair for the determination of a carbohydrate. The analyte can include a protein, a peptide, an amino acid, a nucleotide target, and the like.

An "Expressed Sequence Tag" or "EST" refers to the partial sequence of a cDNA insert which has been made by reverse transcription of mRNA extracted from a tissue, followed by insertion into a vector.

A "transcript image" refers to a table or list giving the quantitative distribution of ESTs in a library and represents the genes active in the tissue from which the library was made.

The present invention provides assays which utilize specific binding members. A "specific binding member," as used herein, is a member of a specific binding pair. That is, two different molecules where one of the molecules through chemical or physical means specifically binds to the second molecule. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, antibodies and antibody fragments, both monoclonal and polyclonal, and complexes thereof, including those formed by recombinant DNA molecules.

The term "hapten," as used herein, refers to a partial antigen or non-protein binding member which is capable of binding to an antibody, but which is not capable of eliciting antibody formation unless coupled to a carrier protein.

A "capture reagent," as used herein, refers to an unlabeled specific binding member which is specific either for the analyte as in a sandwich assay, for the indicator reagent or analyte as in a competitive assay, or for an ancillary specific binding member, which itself is specific for the analyte, as in an indirect assay. The capture reagent can be directly or indirectly bound to a solid phase material before the performance of the assay or during the performance of the assay, thereby enabling the separation of immobilized complexes from the test sample.

The "indicator reagent" comprises a "signal-generating compound" ("label") which is capable of generating and generates a measurable signal detectable by external means, conjugated ("attached") to a specific binding member. "Specific binding member" as used

herein means a member of a specific binding pair. That is, two different molecules where one of the molecules through chemical or physical means specifically binds to the second molecule. In addition to being an antibody member of a specific binding pair, the indicator reagent also can be a member of any specific binding pair, including either hapten-anti-hapten systems such as biotin or anti-biotin, avidin or biotin, a carbohydrate or a lectin, a complementary nucleotide sequence, an effector or a receptor molecule, an enzyme cofactor and an enzyme, an enzyme inhibitor or an enzyme, and the like. An immunoreactive specific binding member can be an antibody, an antigen, or an antibody/antigen complex that is capable of binding either to polypeptide of interest as in a sandwich assay, to the capture reagent as in a competitive assay, or to the ancillary specific binding member as in an indirect assay. When describing probes and probe assays, the term "reporter molecule" may be used. A reporter molecule comprises a signal generating compound as described hereinabove conjugated to a specific binding member of a specific binding pair, such as carbazol or adamantane.

The various "signal-generating compounds" (labels) contemplated include chromogens, catalysts such as enzymes, luminescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as dioxetanes, acridiniums, phenanthridiniums and luminol, radioactive elements, and direct visual labels. Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances.

"Solid phases" ("solid supports") are known to those in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, sheep (or other animal) red blood cells, and Duracytes® (red blood cells "fixed" by pyruvic aldehyde and formaldehyde, available from Abbott Laboratories, Abbott Park, IL) and others. The "solid phase" is not critical and can be selected by one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon chips, sheep (or other suitable animal's) red blood cells and Duracytes® are all suitable examples. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like. A "solid phase", as used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the solid phase can retain an additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding member which is immobilized upon (attached to) the solid phase and which has the ability to

immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent to a solid phase material before the performance of the assay or during the performance of the assay. The solid phase thus can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface of a test tube, microtiter well, sheet, bead, microparticle, chip, sheep (or other suitable animal's) red blood cells, Duracytes® and other configurations known to those of ordinary skill in the art.

It is contemplated and within the scope of the present invention that the solid phase also can comprise any suitable porous material with sufficient porosity to allow access by detection antibodies and a suitable surface affinity to bind antigens. Microporous structure generally are preferred, but materials with gel structure in the hydrated state may be used as well. Such useful solid supports include but are not limited to nitrocellulose and nylon. It is contemplated that such porous solid supports described herein preferably are in the form of sheets of thickness from about 0.01 to 0.5 mm, preferably about 0.1mm. The pore size may vary within wide limits, and preferably is from about 0.025 to 15 microns, especially from about 0.15 to 15 microns. The surface of such supports may be activated by chemical processes which cause covalent linkage of the antigen or antibody to the support. The irreversible binding of the antigen or antibody is obtained, however, in general, by adsorption on the porous material by poorly understood hydrophobic forces. Other suitable solid supports are known in the art.

Reagents.

The present invention provides reagents such as human MMP19 polynucleotide sequences derived from an MMP19 of interest, polypeptides encoded therein, and antibodies developed from these polypeptides. The present invention also provides reagents such as oligonucleotide fragments derived from the disclosed polynucleotides and nucleic acid sequences complementary to the these polynucleotides. The polynucleotides or polypeptides or antibodies of the present invention may be used in the diagnosis, prognosis, and/or treatment of individuals with conditions associated with the expression of the MMP19 gene, such as cancer, arthritis, or inflammation, or to identify a predisposition to these conditions. The sequences disclosed herein represent unique polynucleotides which can be used in assays or for producing a disease specific profile of gene transcription activity.

Selected MMP19 polynucleotides can be used in the methods described herein for the detection of normal or altered gene expression. Such methods may employ the MMP19 polynucleotides disclosed herein or oligonucleotides, fragments or derivatives thereof, or nucleic acid sequences complementary to these polynucleotides.

The polynucleotides disclosed herein, their complementary sequences or fragments of either can be used in assays to detect, amplify or quantify genes, cDNAs or mRNAs relating to cancer, arthritis, or inflammation and associated conditions. They also can be used to identify

an entire or partial coding region which encodes for MMP19 polypeptide. They further can be provided in individual containers in the form of a kit for assays, or provided as individual compositions. If provided in a kit for assays, other suitable reagents such as buffers, conjugates and the like may be included.

The polynucleotide(s) may be in the form of mRNA or DNA. Polynucleotides in the form of DNA, cDNA, genomic DNA, and synthetic DNA are within the scope of the present invention. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding (sense) strand or non-coding (anti-sense) strand. The coding sequence which encodes the polypeptide may be identical to the coding sequence provided herein or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide as the DNA provided herein.

This polynucleotide may include only the coding sequence for the polypeptide, or the coding sequence for the polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence, or the coding sequence for the polypeptide (and optionally additional coding sequence) and non-coding sequence, such as a non-coding sequence 5' and/or 3' of the coding sequence for the polypeptide.

In addition, the invention includes variant polynucleotides containing modifications such as polynucleotide deletions, substitutions or additions; and any polypeptide modification resulting from the variant polynucleotide sequence. A polynucleotide of the present invention also may have a coding sequence which is a naturally occurring allelic variant of the coding sequence provided herein.

In addition, the coding sequence for the polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the polypeptide. The polynucleotides may also encode for a proprotein which is the protein plus additional amino acid residues at the N-terminus. A protein having a prosequence is a proprotein and may in some cases be an inactive form of the protein. Once the prosequence is cleaved an active protein remains. Thus, the polynucleotide of the present invention may encode for a protein, or for a protein having a prosequence or for a protein having both a presequence (leader sequence) and a prosequence.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian

host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein. See, for example, I. Wilson, et al., Cell 37:767 (1984).

It is contemplated that polynucleotides will be considered to hybridize to the sequences provided herein if there is at least 50%, and preferably at least 70% identity between the polynucleotide and the sequence.

Probes constructed according to the polynucleotide sequences of the present invention can be used in various assay methods to provide various types of analysis. For example, such probes can be used in Fluorescent In Situ Hybridization (FISH) technology to perform chromosomal analysis, and used to identify cancer-specific structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR-generated and/or allele specific oligonulcleotides probes, allele specific amplification or by direct sequencing. Probes also can be labeled with radioisotopes, directly- or indirectly- detectable haptens, or fluorescent molecules, and utilized for in situ hybridization studies to evaluate the mRNA expression of the gene comprising the polynucleotide in fixed tissue specimens or cells.

The present invention further relates to an MMP19 polypeptide which has the deduced amino acid sequence as provided herein, as well as fragments, analogs and derivatives of such polypeptide. The polypeptide of the present invention may be a recombinant polypeptide, a natural purified polypeptide or a synthetic polypeptide. The fragment, derivative or analog of the MMP19 polypeptide may be one in which one or more of the amino acid residues is substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; or it may be one in which one or more of the amino acid residues includes a substituent group; or it may be one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or it may be one in which the additional amino acids are fused to the polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are within the scope of the present invention. The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably purified.

Thus, a polypeptide of the present invention may have an amino acid sequence that is identical to that of the naturally occurring polypeptide or that is different by minor variations due to one or more amino acid substitutions. The variation may be a "conservative change" typically in the range of about 1 to 5 amino acids, wherein the substituted amino acid has similar structural or chemical properties, eg, replacement of leucine with isoleucine or threonine with serine. In contrast, variations may include nonconservative changes, eg, replacement of a glycine with a tryptophan. Similar minor variations may also include amino

acid deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without changing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software (DNASTAR Inc., Madison WI).

Preferably, a polypeptide of the present invention is one having at least 40% identity, more preferably, at least 50% identity, even more preferably at least 60% identity and even more preferably, at least 70% identity to SEQ ID NO:10. Such a polypeptide may also possess the ability to cleave at least the substrate described as SEQ ID NO:13.

The present invention also provides an antibody produced by using a purified MMP19 polypeptide of which at least a portion of the polypeptide is encoded by MMP19 polynucleotide selected from the polynucleotides provided herein. These antibodies may be used in the methods provided herein for the detection of MMP19 polypeptides in test samples. The antibody also may be used for therapeutic purposes, for example, in neutralizing the activity of a MMP19 polypeptide in conditions associated with altered or abnormal expression of the MMP19 gene.

This invention also provides teachings as to the production of the polynucleotides and polypeptides provided herein.

Probe Assays

The sequences provided herein may be used to produce probes which can be used in assays for the detection of nucleic acids in test samples. The probes may be designed from conserved nucleotide regions of the polynucleotides of interest or from non-conserved nucleotide regions of the polynucleotide of interest. The design of such probes for optimization in assays is within the skill of the routineer. Generally, nucleic acid probes are developed from non-conserved or unique regions when maximum specificity is desired, and nucleic acid probes are developed from conserved regions when assaying for nucleotide regions that are closely related to, for example, different members of a multigene family or in related species like mouse and man.

The polymerase chain reaction (PCR) is a technique for amplifying a desired nucleic acid sequence (target) contained in a nucleic acid or mixture thereof. In PCR, a pair of primers are employed in excess to hybridize at the outside ends of complementary strands of the target nucleic acid. The primers are each extended by a polymerase using the target nucleic acid as a template. The extension products become target sequences themselves, following dissociation from the original target strand. New primers then are hybridized and extended by a polymerase, and the cycle is repeated to geometrically increase the number of target sequence molecules. PCR is disclosed in U.S. patents 4,683,195 and 4,683,202, which are incorporated herein by reference.

The Ligase Chain Reaction (LCR) is an alternate method for nucleic acid amplification. In LCR, probe pairs are used which include two primary (first and second) and two secondary (third and fourth) probes, all of which are employed in molar excess to target. The first probe hybridizes to a first segment of the target strand and the second probe hybridizes to a second segment of the target strand, the first and second segments being contiguous so that the primary probes abut one another in 5' phosphate-3'hydroxyl relationship, and so that a ligase can covalently fuse or ligate the two probes into a fused product. In addition, a third (secondary) probe can hybridize to a portion of the first probe and a fourth (secondary) probe can hybridize to a portion of the second probe in a similar abutting fashion. Of course, if the target is initially double stranded, the secondary probes also will hybridize to the target complement in the first instance. Once the ligated strand of primary probes is separated from the target strand, it will hybridize with the third and fourth probes which can be ligated to form a complementary, secondary ligated product. It is important to realize that the ligated products are functionally equivalent to either the target or its complement. By repeated cycles of hybridization and ligation, amplification of the target sequence is achieved. This technique is described more completely in EP-A- 320 308 to K. Backman published June 16, 1989 and EP-A-439 182 to K. Backman et al, published July 31, 1991, both of which are incorporated herein by reference.

For amplification of mRNAs, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Patent No. 5,322,770, which is incorporated herein by reference; or reverse transcribe mRNA into cDNA followed by asymmetric gap ligase chain reaction (RT-AGLCR) as described by R. L. Marshall, et al., PCR Methods and Applications 4: 80-84 (1994), which also is incorporated herein by reference.

Other known amplification methods which can be utilized herein include but are not limited to the so-called "NASBA" or "3SR" technique described in PNAS USA 87:1874-1878 (1990) and also described in Nature 350 (No. 6313):91-92 (1991); Q-beta amplification as described in published European Patent Application (EPA) No. 4544610; strand displacement amplification (as described in G. T. Walker et al., Clin. Chem. 42:9-13 [1996]) and European Patent Application No. 684315; and target mediated amplification, as described by PCT Publication WO 9322461.

In one embodiment, the present invention generally comprises the steps of contacting a test sample suspected of containing a target polynucleotide sequence with amplification reaction reagents comprising an amplification primer, and a detection probe that can hybridize with an internal region of the amplicon sequences. Probes and primers employed according to the method herein provided are labeled with capture and detection labels wherein probes are

labeled with one type of label and primers are labeled with the other type of label. Additionally, the primers and probes are selected such that the probe sequence has a lower melt temperature than the primer sequences. The amplification reagents, detection reagents and test sample are placed under amplification conditions whereby, in the presence of target sequence, copies of the target sequence (an amplicon) are produced. In the usual case, the amplicon is double stranded because primers are provided to amplify a target sequence and its complementary strand. The double stranded amplicon then is thermally denatured to produce single stranded amplicon members. Upon formation of the single stranded amplicon members, the mixture is cooled to allow the formation of complexes between the probes and single stranded amplicon members.

As the single stranded amplicon sequences and probe sequences are cooled, the probe sequences preferentially bind the single stranded amplicon members. This finding is counterintuitive given that the probe sequences are generally selected to be shorter than the primer sequences and therefore have a lower melt temperature than the primers. Accordingly, the melt temperature of the amplicon produced by the primers should also have a higher melt temperature than the probes. Thus, as the mixture is cooled, the re-formation of the double stranded amplicon is expected although as previously stated, this is not the case. Instead, the probes are found to preferentially bind the single stranded amplicon members. Moreover, this preference of probe/single stranded amplicon binding exists even when the primer sequences are added in excess of the probes.

After the probe/single stranded amplicon member hybrids are formed, they are detected. Standard heterogeneous assay formats are suitable for detecting the hybrids using the detection labels and capture labels present on the primers and probes. The hybrids can be bound to a solid phase reagent by virtue of the capture label and detected by virtue of the detection label. In cases where the detection label is directly detectable, the presence of the hybrids on the solid phase can be detected by causing the label to produce a detectable signal, if necessary, and detecting the signal. In cases where the label is not directly detectable, the captured hybrids can be contacted with a conjugate, which generally comprises a binding member attached to a directly detectable label. The conjugate becomes bound to the complexes and the conjugates presence on the complexes can be detected with the directly detectable label. Thus, the presence of the hybrids on the solid phase reagent can be determined. Those skilled in the art will recognize that wash steps may be employed to wash away unhybridized amplicon or probe as well as unbound conjugate.

A test sample is typically anything suspected of containing a target sequence. Test samples can be prepared using methodologies well known in the art such as by obtaining a specimen from an individual and, if necessary, disrupting any cells contained therein to release target nucleic acids. Although the target sequence is described as single stranded, it also is

contemplated to include the case where the target sequence is actually double stranded but is merely separated from its complement prior to hybridization with the amplification primer sequences. In the case where PCR is employed in this method, the ends of the target sequences are usually known. In cases where LCR or a modification thereof is employed in the preferred method, the entire target sequence is usually known. Typically, the target sequence is a nucleic acid sequence such as, for example, RNA or DNA.

The method provided herein can include well known amplification reactions that utilize thermal cycle reaction mixtures, particularly PCR and GLCR. Amplification reactions typically employ primers to repeatedly generate copies of a target nucleic acid sequence, which target sequence is usually a small region of a much larger nucleic acid sequence. Primers are themselves nucleic acid sequences that are complementary to regions of a target sequence. Under amplification conditions, these primers hybridize or bind to the complementary regions of the target sequence. Copies of the target sequence typically are generated by the process of primer extension and/or ligation which utilizes enzymes with polymerase or ligase activity, separately or in combination, to add nucleotides to the hybridized primers and/or ligate adjacent probe pairs. The nucleotides that are added to the primers or probes, as monomers or preformed oligomers, are also complementary to the target sequence. Once the primers or probes have been sufficiently extended and/or ligated they are separated from the target sequence, for example, by heating the reaction mixture to a "melt temperature" which is one in which complementary nucleic acid strands dissociate. Thus, a sequence complementary to the target sequence is formed.

A new amplification cycle then can take place to further amplify the number of target sequences by separating any double stranded sequences, allowing primers or probes to hybridize to their respective targets, extending and/or ligating the hybridized primers or probes and re-separating. The complementary sequences that are generated by amplification cycles can serve as templates for primer extension or filling the gap of two probes to further amplify the number of target sequences. Typically, a reaction mixture is cycled between 20 and 100 times, more typically, a reaction mixture is cycled between 25 and 50 times. The numbers of cycles can be determined by the routineer. In this manner, multiple copies of the target sequence and its complementary sequence are produced. Thus, primers initiate amplification of the target sequence when it is present under amplification conditions.

Generally, two primers which are complementary to a portion of a target strand and its complement are employed in PCR. For LCR, four probes, two of which are complementary to a target sequence and two of which are similarly complementary to the targets complement, are generally employed. In addition to the primer sets and enzymes previously mentioned, a nucleic acid amplification reaction mixture may also comprise other reagents which are well known and include but are not limited to: enzyme cofactors such as manganese; magnesium;

salts; nicotinamide adenine dinucleotide (NAD); and deoxynucleotide triphosphates (dNTPs) such as for example deoxyadenine triphosphate, deoxyguanine triphosphate, deoxycytosine triphosphate and deoxythymine triphosphate.

While the amplification primers initiate amplification of the target sequence, in some cases, the detection (or hybridization) probe is not involved in amplification. Detection probes are generally nucleic acid sequences or uncharged nucleic acid analogs such as, for example, peptide nucleic acids which are disclosed in International Patent Application WO 92/20702; morpholino analogs which are described in U.S. Patents Nos 5,185,444, 5,034,506, and 5,142,047; and the like. Depending upon the type of label carried by the probe, the probe is employed to capture or detect the amplicon generated by the amplification reaction. The probe is not involved in amplification of the target sequence and therefore may have to be rendered "non-extendable" in that additional dNTPs cannot be added to the probe. In and of themselves analogs usually are non-extendable and nucleic acid probes can be rendered non-extendable by modifying the 3' end of the probe such that the hydroxyl group is no longer capable of participating in elongation. For example, the 3' end of the probe can be functionalized with the capture or detection label to thereby consume or otherwise block the hydroxyl group. Alternatively, the 3' hydroxyl group simply can be cleaved, replaced or modified. U.S. Patent Application Serial No. 07/049,061 filed April 19, 1993 and incorporated herein by reference describes modifications which can be used to render a probe non-extendable.

Accordingly, the ratio of primers to probes is not important. Thus, either the probes or primers can be added to the reaction mixture in excess whereby the concentration of one would be greater than the concentration of the other. Alternatively, primers and probes can be employed in equivalent concentrations. Preferably, however, the primers are added to the reaction mixture in excess of the probes. Thus, primer to probe ratios of, for example, 5:1 and 20:1 are preferred.

While the length of the primers and probes can vary, the probe sequences are selected such that they have a lower melt temperature than the primer sequences. Hence, the primer sequences are generally longer than the probe sequences. Typically, the primer sequences are in the range of between 20 and 50 nucleotides long, more typically in the range of between 20 and 30 nucleotides long. The typical probe is in the range of between 10 and 25 nucleotides long.

Alternatively, a probe may be involved in the amplifying a target sequence, via a process known as "nested PCR". In nested PCR, the probe has characteristics which are similar to those of the first and second primers normally used for amplification (such as length, melting temperature etc.) and as such, may itself serve as a primer in an amplification reaction. Generally in nested PCR, a first pair of primers (P_1 and P_2) are employed to form primary extension products. One of the primary primers (for example, P_1) may optionally be a capture

primer (i.e. linked to a member of a first reactive pair), whereas the other primary primer (P_2) is not. A secondary extension product is then formed using the P_1 primer and a probe ($P_{2'}$) which may also have a capture type label (such as a member of a second reactive pair) or a detection label at its 5' end. The probe is complimentary to and hybridizes at a site on the template near or adjacent (but not overlapping) the site where the 3' terminus of P_2 would hybridize if it was still in solution. Thus, a labeled primer/probe set generates a secondary product which is shorter than the primary extension product. Furthermore, the secondary product may be detected either on the basis of its size or via its labeled ends (by detection methodologies well known to those of ordinary skill in the art). In this process, probe and primers are generally employed in equivalent concentrations.

Various methods for synthesizing primers and probes are well known in the art. Similarly, methods for attaching labels to primers or probes are also well known in the art. For example, it is a matter of routine to synthesize desired nucleic acid primers or probes using conventional nucleotide phosphoramidite chemistry and instruments available from Applied Biosystems, Inc., (Foster City, CA), Dupont (Wilmington, DE), or Milligen (Bedford MA). Many methods have been described for labeling oligonucleotides such as the primers or probes of the present invention. Enzo Biochemical (New York, NY) and Clontech (Palo Alto, CA) both have described and commercialized probe labeling techniques. For example, a primary amine can be attached to a 3' oligo terminus using 3'-Amine-ON CPG™ (Clontech, Palo Alto, CA). Similarly, a primary amine can be attached to a 5' oligo terminus using Aminomodifier II® (Clontech). The amines can be reacted to various haptens using conventional activation and linking chemistries. In addition, copending applications US. Serial Nos. 625,566, filed December 11, 1990 and 630,908, filed December 20, 1990, which are each incorporated herein by reference, teach methods for labeling probes at their 5' and 3' termini, respectively. Publications WO92/10505, published 25 June 1992 and WO 92/11388 published 9 July 1992 teach methods for labeling probes at their 5' and 3' ends, respectively. According to one known method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. See, for example, N.T. Thuong et al., Tet. Letters 29(46):5905-5908 (1988); or J. S. Cohen et al., published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL-7-246,688) (1989). Preferably, probes are labeled at their 3' and 5' ends.

Capture labels are carried by the primers or probes and can be a specific binding member which forms a binding pair with the solid phase reagent's specific binding member. It will be understood, of course that the primer or probe itself may serve as the capture label. For example, in the case where a solid phase reagent's binding member is a nucleic acid sequence, it may be selected such that it binds a complementary portion of the primer or probe to thereby immobilize the primer or probe to the solid phase. In cases where the probe itself

serves as the binding member, those skilled in the art will recognize that the probe will contain a sequence or "tail" that is not complementary to the single stranded amplicon members. In the case where the primer itself serves as the capture label, at least a portion of the primer will be free to hybridize with a nucleic acid on a solid phase because the probe is selected such that it is not fully complementary to the primer sequence.

Generally, probe/single stranded amplicon member complexes can be detected using techniques commonly employed to perform heterogeneous immunoassays. Preferably, in this embodiment, detection is performed according to the protocols used by the commercially available Abbott LCx® instrumentation (Abbott Laboratories, Abbott Park, IL).

The primers and probes disclosed herein are useful in typical PCR assays, wherein the test sample is contacted with a pair of primers, amplification is performed, the hybridization probe is added, and detection is performed.

Another method provided by the present invention comprises contacting a test sample with a plurality of polynucleotides wherein at least one polynucleotide is provided herein, hybridizing the test sample with the plurality of polynucleotides and detecting the hybridization complexes. The hybridization complexes are identified and quantitated to compile a profile which is indicative of cancer, metastases, arthritis or inflammatory conditions. Expressed RNA sequences may further be detected by reverse transcription and amplification of the DNA product by procedures well-known in the art, including polymerase chain reaction (PCR).

Antisense and Gene Therapy.

The present invention also encompasses the use of gene therapy methods for the introduction of anti-sense MMP19 gene derived molecules such as polynucleotides or oligonucleotides of the present invention into patients having conditions (such as cancer, arthritis or inflammation) that are associated with bnormal expression of MMP19 polynucleotides. These molecules, including antisense RNA and DNA fragments and ribozymes, are designed to inhibit the translation of a MMP19 polynucleotide mRNA, and may be used therapeutically in the treatment of conditions associated with altered or abnormal expression of the MMP19 polynucleotide.

Alternatively, the oligonucleotides described above can be delivered to cells by procedures in the art such that the anti-sense RNA or DNA is subsequently expressed *in vivo* whereby it inhibits production of MMP19 polypeptide in the manner described above. Antisense constructs to MMP19 polynucleotide, therefore, reverse the action of MMP19 transcripts and may be used for treating cancer, arthritis, inflammation and related disease states. These antisense constructs may also be used to treat tumor metastases.

Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide

sequence, which encodes for the polypeptide of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription, thereby preventing transcription and the production of MMP19 polypeptide. For triple helix, see, for example, Lee et al, Nucl. Acids Res. 6:3073 (1979); Cooney et al, Science 241:456 (1988); and Dervan et al, Science 251:1360 (1991) The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of an mRNA molecule into the MMP19 polypeptide. For antisense, see, for example, Okano, J. Neurochem. 56:560 (1991); and "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression", CRC Press, Boca Raton, Fla. (1988). Antisense oligonucleotides act with greater efficacy when modified to contain artificial internucleotide linkages which render the molecule resistant to nucleolytic cleavage. Such artificial internucleotide linkages include but are not limited to methylphosphonate, phosphorothiolate and phosphoroamydate internucleotide linkages.

The present invention also encompasses gene therapy whereby the gene encoding MMP19 is regulated in a patient. Various methods of transferring or delivering DNA to cells for expression of the gene product protein, otherwise referred to as gene therapy, are disclosed in "Gene Transfer into Mammalian Somatic Cells *in vivo*", N. Yang, Crit. Rev. Biotechn. 12(4): 335-356 (1992), which is hereby incorporated by reference. Gene therapy encompasses incorporation of DNA sequences into somatic cells or germ line cells for use in either *ex vivo* or *in vivo* therapy. Gene therapy functions to replace genes, augment normal or abnormal gene function, and to combat infectious diseases and other pathologies.

Strategies for treating medical problems with gene therapy include therapeutic strategies such as identifying the defective gene and then adding a functional gene to either replace the function of the defective gene or to augment a slightly functional gene; or prophylactic strategies, such as adding a gene which encodes a protein product that will treat the condition or that will make the tissue or organ more susceptible to a treatment regimen.

Many protocols for transfer of MMP19 DNA or MMP19 regulatory sequences are envisioned in this invention. Transfection of promoter sequences, other than one specifically associated with MMP19, or other sequences which would increase production of MMP19 protein are also envisioned as methods of gene therapy. An example of this technology is found in Transkaryotic Therapies, Inc., of Cambridge, Massachusetts, using homologous recombination to insert a "genetic switch" that turns on an erythropoietin gene in cells. See Genetic Engineering News, April 15, 1994. Such "genetic switches" could be used to activate MMP19 (or a MMP19 receptor) in cells not normally expressing these proteins.

Gene transfer methods for gene therapy fall into three broad categories: (1) physical (e.g., electroporation, direct gene transfer and particle bombardment), (2) chemical (e.g. lipid-based carriers and other non-viral vectors) and (3) biological (e.g. virus derived vectors). For

example, non-viral vectors such as liposomes coated with DNA may be directly injected intravenously into the patient. It is believed that the liposome/DNA complexes are concentrated in the liver where they deliver the DNA to macrophages and Kupffer cells. Additionally, vectors or the "naked" DNA of the gene may be directly injected into the desired organ, tissue or tumor for targeted delivery of the therapeutic DNA.

Gene therapy methodologies can also be described by delivery site. Fundamental ways to deliver genes include *ex vivo* gene transfer, *in vivo* gene transfer, and in vitro gene transfer. In *ex vivo* gene transfer, cells are taken from the patient and grown in cell culture. The DNA is transfected into the cells, the transfected cells are expanded in number and then reimplanted in the patient. In *in vitro* gene transfer, the transformed cells are cells growing in culture, such as tissue culture cells, and not particular cells from a particular patient. These "laboratory cells" are transfected, the transfected cells are selected and expanded for either implantation into a patient or for other uses. *In vivo* gene transfer involves introducing the DNA into the cells of the patient when the cells are within the patient. All three of the broad based categories described above may be used to achieve gene transfer *in vivo*, *ex vivo*, and *in vitro*.

Mechanical (i.e. physical) methods of DNA delivery can be achieved by direct injection of DNA, such as microinjection of DNA into germ or somatic cells, pneumatically delivered DNA-coated particles, such as the gold particles used in a "gene gun," and inorganic chemical approaches such as calcium phosphate transfection. It has been found that physical injection of plasmid DNA into muscle cells yields a high percentage of cells which are transfected and have a sustained expression of marker genes. The plasmid DNA may or may not integrate into the genome of the cells. Non-integration of the transfected DNA would allow the transfection and expression of gene product proteins in terminally differentiated, non-proliferative tissues for a prolonged period of time without fear of mutational insertions, deletions, or alterations in the cellular or mitochondrial genome. Long-term, but not necessarily permanent, transfer of therapeutic genes into specific cells may provide treatments for genetic diseases or for prophylactic use. The DNA could be reinjected periodically to maintain the gene product level without mutations occurring in the genomes of the recipient cells. Non-integration of exogenous DNAs may allow for the presence of several different exogenous DNA constructs within one cell with all of the constructs expressing various gene products.

Particle-mediated gene transfer may also be employed for injecting DNA into cells, tissues and organs. With a particle bombardment device, or "gene gun," a motive force is generated to accelerate DNA-coated high density particles (such as gold or tungsten) to a high velocity that allows penetration of the target organs, tissues or cells. Electroporation for gene transfer uses an electrical current to make cells or tissues susceptible to electroporation-mediated gene transfer. A brief electric impulse with a given field strength is used to increase the permeability of a membrane in such a way that DNA molecules can penetrate into the cells.

The techniques of particle-mediated gene transfer and electroporation are well known to those of ordinary skill in the art.

Chemical methods of gene therapy involve carrier mediated gene transfer through the use of fusogenic lipid vesicles such as liposomes or other vesicles for membrane fusion. A carrier harboring a DNA of interest can be conveniently introduced into body fluids or the bloodstream and then site specifically directed to the target organ or tissue in the body. Liposomes, for example, can be developed which are cell specific or organ specific. The foreign DNA carried by the liposome thus will be taken up by those specific cells. Injection of immunoliposomes that are targeted to a specific receptor on certain cells can be used as a convenient method of inserting the DNA into the cells bearing the receptor. Another carrier system that has been used is the asialoglycoprotein/polylysine conjugate system for carrying DNA to hepatocytes for *in vivo* gene transfer.

Transfected DNA may also be complexed with other kinds of carriers so that the DNA is carried to the recipient cell and then resides in the cytoplasm or in the nucleoplasm of the recipient cell. DNA can be coupled to carrier nuclear proteins in specifically engineered vesicle complexes and carried directly into the nucleus.

Carrier mediated gene transfer may also involve the use of lipid-based proteins which are not liposomes. For example, lipofectins and cytofectins are lipid-based positive ions that bind to negatively charged DNA, forming a complex that can ferry the DNA across a cell membrane. Another method of carrier mediated gene transfer involves receptor-based endocytosis. In this method, a ligand (specific to a cell surface receptor) is made to form a complex with a gene of interest and then injected into the bloodstream; target cells that have the cell surface receptor will specifically bind the ligand and transport the ligand-DNA complex into the cell.

Biological gene therapy methodologies usually employ viral vectors to insert genes into cells. The term "vector" as used herein in the context of biological gene therapy means a carrier that can contain or associate with specific polynucleotide sequences and which functions to transport the specific polynucleotide sequences into a cell. The transfected cells may be cells derived from the patient's normal tissue, the patient's diseased tissue, or may be non-patient cells. Examples of vectors include plasmids and infective microorganisms such as viruses, or non-viral vectors such as the ligand-DNA conjugates, liposomes, and lipid-DNA complexes discussed above.

It may be desirable that a recombinant DNA molecule comprising a MMP19 DNA sequence is operatively linked to an expression control sequence to form an expression vector capable of expressing MMP19. Alternatively, gene regulation of MMP19 may be accomplished by administering proteins that bind to control regions associated with the

MMP19 gene, or its corresponding RNA transcript to modify the rate of transcription or translation.

Viral vectors that have been used for gene therapy protocols include but are not limited to, retroviruses, other RNA viruses such as poliovirus or Sindbis virus, adenovirus, adeno-associated virus, herpes viruses, SV 40, vaccinia and other DNA viruses. Replication-defective murine retroviral vectors are the most widely utilized gene transfer vectors. Murine leukemia retroviruses are composed of a single strand RNA complexed with a nuclear core protein and polymerase (pol) enzymes, encased by a protein core (gag) and surrounded by a glycoprotein envelope (env) that determines host range. The genomic structure of retroviruses include the gag, pol, and env genes enclosed at by 5' and 3' long terminal repeats (LTR). Retroviral vector systems exploit the fact that a minimal vector containing the 5' and 3' LTRs and the packaging signal are sufficient to allow vector packaging, infection and integration into target cells providing that the viral structural proteins are supplied *in trans* in the packaging cell line. Fundamental advantages of retroviral vectors for gene transfer include efficient infection and gene expression in most cell types, precise single copy vector integration into target cell chromosomal DNA, and ease of manipulation of the retroviral genome. For example, altered retrovirus vectors have been used in *ex vivo* methods to introduce genes into peripheral and tumor-infiltrating lymphocytes, hepatocytes, epidermal cells, myocytes, or other somatic cells (which may then introduced into the patient to provide the gene product from the inserted DNA).

The adenovirus is composed of linear, double stranded DNA complexed with core proteins and surrounded with capsid proteins. Advances in molecular virology have led to the ability to exploit the biology of these organisms to create vectors capable of transducing novel genetic sequences into target cells *in vivo* . Adenoviral-based vectors will express gene product peptides at high levels. Adenoviral vectors have high efficiencies of infectivity, even with low titers of virus. Additionally, the virus is fully infective as a cell free virion so injection of producer cell lines are not necessary. Another potential advantage to adenoviral vectors is the ability to achieve long term expression of heterologous genes *in vivo*.

Viral vectors have also been used to insert genes into cells using *in vivo* protocols. To direct tissue-specific expression of foreign genes, cis-acting regulatory elements or promoters that are known to be tissue specific can be used. Alternatively, this can be achieved using *in situ* delivery of DNA or viral vectors to specific anatomical sites *in vivo*. For example, gene transfer to blood vessels *in vivo* was achieved by implanting *in vitro* transduced endothelial cells in chosen sites on arterial walls. The virus infected surrounding cells which also expressed the gene product. A viral vector can be delivered directly to the *in vivo* site, by a catheter for example, thus allowing only certain areas to be infected by the virus, and providing long-term, site specific gene expression. *In vivo* gene transfer using retrovirus

vectors has also been demonstrated in mammary tissue and hepatic tissue by injection of the altered virus into blood vessels leading to the organs.

Drug Screening.

The present invention also provides a method of screening a plurality of compounds for specific binding to a MMP19 polypeptide, or any fragment thereof, to identify at least one compound which specifically binds the MMP19 polypeptide. Such a method comprises the steps of providing at least one compound; combining the MMP19 polypeptide with each compound under suitable conditions for a time sufficient to allow binding; and detecting MMP19 polypeptide binding to each compound.

The present invention also provides a method of screening a plurality of compounds for inhibition of the activity of a MMP19 polypeptide, or any fragment thereof, to identify at least one compound which specifically inhibits the activity of the MMP19 polypeptide. Such a method comprises the steps of providing at least one compound; combining the MMP19 polypeptide with each compound under suitable conditions for a time sufficient to allow interaction or binding; and detecting MMP19 polypeptide inhibition by each compound.

The polypeptide or peptide fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids which can express the polypeptide or peptide fragment. Drugs may be screened against such transformed cells in competitive binding or enzymatic inhibition assays. For example, the formation of complexes between a polypeptide and the agent being tested can be measured in either viable or fixed cells.

The present invention thus provides methods of screening for drugs or any other agent which can be used to treat conditions associated with cancer, arthritis, or inflammation resulting from abnormal MMP19 production. These methods comprise contacting the drug with a polypeptide or fragment thereof and assaying for either the presence of a complex between the agent and the polypeptide, or for the presence of a complex between the polypeptide and the cell. In competitive binding assays, the polypeptide typically is labeled. After suitable incubation, free (or uncomplexed) polypeptide or fragment thereof is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular drug to bind to polypeptide or to interfere with the polypeptide/cell complex.

The present invention also encompasses the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptide specifically compete with a test drug for binding to the polypeptide or fragment thereof. In this manner, the antibodies can be used to detect the presence of any polypeptide in the test sample which shares one or more antigenic determinants with a polypeptide provided herein.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to at least one polypeptide disclosed herein. Briefly, large numbers of different small peptide test compounds are synthesized on a solid phase, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptide and washed. Polypeptide thus bound to the solid phase is detected by methods well-known in the art. Purified polypeptide can also be coated directly onto plates for use in the drug screening techniques described herein. In addition, non-neutralizing antibodies can be used to capture the polypeptide and immobilize it on the solid support. See, for example, EP 84/03564, published on September 13, 1984, which is incorporated herein by reference.

Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of the small molecules including agonists, antagonists, or inhibitors with which they interact. Such structural analogs can be used to fashion drugs which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a polypeptide *in vivo*. J. Hodgson, Bio/Technology 9:19-21 (1991), incorporated herein by reference.

For example, in one approach, the three-dimensional structure of a polypeptide, or of a polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous polypeptide-like molecules or to identify efficient inhibitors

Useful examples of rational drug design may include molecules which have improved activity or stability as shown by S. Braxton et al., Biochemistry 31:7796-7801 (1992), or which act as inhibitors, agonists, or antagonists of native peptides as shown by S. B. P. Athauda et al., J Biochem. (Tokyo) 113 (6):742-746 (1993), incorporated herein by reference.

It also is possible to isolate a target-specific antibody, selected by an assay as described hereinabove, and then to determine its crystal structure. In principle this approach yields a pharmacophore upon which subsequent drug design can be based. It further is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies ("anti-ids") to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-id is an analog of the original receptor. The anti-id then could be used to identify and isolate peptides from banks of chemically or biologically produced peptides.

The isolated peptides then can act as the pharmacophore (that is, a prototype pharmaceutical drug).

A sufficient amount of a recombinant polypeptide of the present invention may be made available to perform analytical studies such as X-ray crystallography. In addition, knowledge of the polypeptide amino acid sequence which are derivable from the nucleic acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

Antibodies specific to the MMP19 polypepeptide may further be used to inhibit the biological action of the polypeptide by binding to the polypeptide. In this manner, the antibodies may be used in therapy, for example, to treat cancer and its metastases.

Further, such antibodies can detect the presence or absence of the MMP19 polypeptide and, therefore, are useful as diagnostic markers for the diagnosis of cancers, arthritis, and inflammatory conditions. Such antibodies may also function as a diagnostic marker for these conditions. The present invention also is directed to antagonists and inhibitors of the polypeptides of the present invention. The antagonists and inhibitors are those which inhibit or eliminate the function of the polypeptide. Thus, for example, an antagonist may bind to a polypeptide of the present invention and inhibit or eliminate its function. The antagonist, for example, could be an antibody against the polypeptide which eliminates the activity of the MMP19 polypeptide by binding to the MMP19 polypeptide, or in some cases the antagonist may be an oligonucleotide. Examples of small molecule inhibitors include but are not limited to small peptides or peptide-like molecules.

The antagonists and inhibitors may be employed as a composition with a pharmaceutically acceptable carrier, including but not limited to saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. Administration of MMP19 polypeptide inhibitors are preferably systemic. The present invention also provides an antibody which inhibits the action of such polypeptide.

Recombinant Technology.

The present invention provides host cells and expression vectors comprising polynucleotides of the present invention and methods for the production of polypeptides they encode. Such methods comprise culturing the host cells under conditions suitable for the expression of the MMP19 polynucleotide and recovering the MMP19 polypeptide from the cell culture.

The present invention also provides vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the present invention and the production of polypeptides of the present invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be a cloning vector or an expression vector. The vector may be in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the MMP19 genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotide of the present invention may be employed for producing a polypeptide by recombinant techniques. Thus, the polynucleotide sequence may be included in any one of a variety of expression vehicles, in particular vectors or plasmids for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other plasmid or vector may be used so long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into appropriate restriction endonuclease sites by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art. The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. Representative examples of such promoters include but are not limited to LTR or SV40 promoter, the E. coli lac or trp, the phage lambda P sub L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. In addition, the expression vectors preferably contain a gene to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein. As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Salmonella typhimurium; Streptomyces sp.; fungal cells, such as yeast; insect cells such as Drosophila and Sf9; animal cells such as CHO, COS or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings provided herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pSPORT1 (Life Technologies, Gaithersburg, MD), pQE70, pQE60, pQE-9 (Qiagen) pBs, phagescript, psiX174, pBluescript SK, pBsKS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as it is replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, SP6, T7, gpt, lambda P sub R, P sub L and trp. Eukaryotic promoters include cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention provides host cells containing the above-described construct. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (L. Davis et al., "Basic Methods in Molecular Biology", 2nd edition, Appleton and Lang, Paramount Publishing, East Norwalk, CT (1994).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor, N.Y., 1989), which is hereby incorporated by reference.

Transcription of a DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin (bp 100 to 270), a cytomegalovirus early promoter enhancer, a polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), alpha factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a routine matter of choice.

Useful expression vectors for bacterial use comprise a selectable marker and bacterial origin of replication derived from plasmids comprising genetic elements of the well-known cloning vector pBR322 (ATCC 37017). Other vectors include but are not limited to PKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction), and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression

of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents; such methods are well-known to the ordinary artisan.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts described by Gluzman, Cell 23:175 (1981), and other cell lines capable of expressing a compatible vector, such as the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Representative, useful vectors include pRc/CMV and pcDNA3 (available from Invitrogen, San Diego, CA).

MMP19 polypeptide is recovered and purified from recombinant cell cultures by known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography or lectin chromatography. It is preferred to have low concentrations (approximately 0.1-5 mM) of calcium ion present during purification (Price, et al., J. Biol. Chem. 244:917 [1969]). Protein refolding steps can be used, as necessary, in completing configuration of the protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be naturally purified products expressed from a high expressing cell line, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated. The polypeptides of the invention may also include an initial methionine amino acid residue.

The starting plasmids can be constructed from available plasmids in accord with published, known procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

The following is the general procedure for the isolation and analysis of cDNA clones. In a particular embodiment disclosed herein, an EST homologous to a portion of human MMPs was identified by comparison with a consensus sequence to human MMPs.

The cDNA insert was sequenced in entirety, analyzed in detail set forth in the Examples and is disclosed in the Sequence Listing as SEQ NO: 4. These polynucleotides may contain an entire open reading frame with or without associated regulatory sequences for a particular gene, or they may encode only a portion of the gene of interest. This is attributed to the fact that many genes are several hundred, and sometimes several thousand, bases in length and, with current technology, cannot be cloned in their entirety because of vector limitations, incomplete reverse transcription of the first strand, or incomplete replication of the second strand. Contiguous, secondary clones containing additional nucleotide sequence may be obtained using a variety of methods known to those of skill in the art.

Methods for DNA sequencing are well known in the art. Conventional enzymatic methods employ DNA polymerase, Klenow fragment, Sequenase (US Biochemical Corp, Cleveland, OH) or Taq polymerase to extend DNA chains from an oligonucleotide primer annealed to the DNA template of interest. Methods have been developed for the use of both single-stranded and double-stranded templates. The chain termination reaction products may be electrophoresed on urea/polyacrylamide gels and detected either by autoradiography (for radionucleotide labeled precursors) or by fluorescence (for fluorescent-labeled precursors). Recent improvements in mechanized reaction preparation, sequencing and analysis using the fluorescent detection method have permitted expansion in the number of sequences that can be determined per day using machines such as the Applied Biosystems 377 DNA Sequencers (Applied Biosystems, Foster City, CA).

The reading frame of the nucleotide sequence can be ascertained by several types of analyses. First, reading frames contained within the coding sequence can be analyzed for the presence of start codon ATG and stop codons TGA, TAA or TAG. Typically, one reading frame will continue throughout the major portion of a cDNA sequence while the other two reading frames tend to contain numerous stop codons. In such cases reading frame determination is straightforward. In other more difficult cases, further analysis is required.

Algorithms have been created to analyze the occurrence of individual nucleotide bases at each putative codon triplet. See, for example J. W. Fickett, Nuc Acids Res 10:5303 (1982). Coding DNA for particular organisms (bacteria, plants, and animals) tends to contain certain nucleotides within certain triplet periodicities, such as a significant preference for pyrimidines in the third codon position. These preferences have been incorporated into widely available software which can be used to determine coding potential (and frame) of a given stretch of DNA. The algorithm-derived information combined with start/stop codon information can be used to determine proper frame with a high degree of certainty. This, in turn, readily permits cloning of the sequence in the correct reading frame into appropriate expression vectors.

The nucleic acid sequences disclosed herein may be joined to a variety of other polynucleotide sequences and vectors of interest by means of well established recombinant DNA techniques. See J. Sambrook et al., *supra*. Vectors of interest include cloning vectors, such as plasmids, cosmids, phage derivatives, phagemids, as well as sequencing, replication, and expression vectors, and the like. In general, such vectors contain an origin of replication functional in at least one organism, convenient restriction endonuclease digestion sites, and selectable markers appropriate for particular host cells. The vectors can be transferred by a variety of means known to those of skill in the art into suitable host cells which then produce the desired DNA, RNA or polypeptides.

Occasionally, sequencing or random reverse transcription errors will mask the presence of the appropriate open reading frame or regulatory element. In such cases, it is possible to determine the correct reading frame by attempting to express the polypeptide and determining the amino acid sequence by standard peptide mapping and sequencing techniques. See, F. M. Ausubel, et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY (1989). Additionally, the actual reading frame of a given nucleotide sequence may be determined by transfection of host cells with vectors containing all three potential reading frames. Only those cells with the nucleotide sequence in the correct reading frame will produce a peptide of the predicted length.

The nucleotide sequences provided herein have been prepared by current, state-of-the-art, automated methods and as such may contain unidentified nucleotides. These will not present a problem to those skilled in the art who wish to practice the invention. Several methods employing standard recombinant techniques, described in J. Sambrook (supra) or periodic updates thereof, may be used to complete the missing sequence information. The same techniques used for obtaining a full length sequence, as described herein, may be used to obtain nucleotide sequence.

Expression of a particular cDNA may be accomplished by subcloning the cDNA into an appropriate expression vector and transfecting this vector into an appropriate expression host. The cloning vector used for the generation of the cDNA library can be used for transcribing mRNA of a particular cDNA and contains a promoter for beta-galactosidase, an amino-terminal met and the subsequent seven amino acid residues of beta-galactosidase. Immediately following these eight residues is an engineered bacteriophage promoter useful for artificial priming and transcription and a number of unique restriction sites, including EcoR I, for cloning. The vector can be transfected into an appropriate host strain of E. coli.

Induction of the isolated bacterial strain with isopropylthiogalactoside (IPTG) using standard methods will produce a fusion protein which contains the first seven residues of beta-galactosidase, about 15 residues of linker, and the peptide encoded within the cDNA. Since cDNA clone inserts are generated by an essentially random process, there is one chance in

three that the included cDNA will lie in the correct frame for proper translation. If the cDNA is not in the proper reading frame, the correct frame can be obtained by deletion or insertion of an appropriate number of bases by well known methods including in vitro mutagenesis, digestion with exonuclease III or mung bean nuclease, or oligonucleotide linker inclusion.

The cDNA can be shuttled into other vectors known to be useful for expression of protein in specific hosts. Oligonucleotide primers containing cloning sites and segments of DNA sufficient to hybridize to stretches at both ends of the target cDNA can be synthesized chemically by standard methods. These primers can then be used to amplify the desired gene segments by PCR. The resulting new gene segments can be digested with appropriate restriction enzymes under standard conditions and isolated by gel electrophoresis. Alternately, similar gene segments can be produced by digestion of the cDNA with appropriate restriction enzymes and filling in the missing gene segments with chemically synthesized oligonucleotides. Segments of the coding sequence from more than one gene can be ligated together and cloned in appropriate vectors to optimize expression of recombinant sequence.

Suitable expression hosts for such chimeric molecules include but are not limited to, mammalian cells such as Chinese Hamster Ovary (CHO) and human 293 cells, insect cells such as Sf9 cells, yeast cells such as Saccharomyces cerevisiae, and bacteria such as E. coli. For each of these cell systems, a useful expression vector may also include an origin of replication to allow propagation in bacteria and a selectable marker such as the beta-lactamase antibiotic resistance gene to allow selection in bacteria. In addition, the vectors may include a second selectable marker such as the neomycin phosphotransferase gene to allow selection in transfected eukaryotic host cells. Vectors for use in eukaryotic expression hosts may require the addition of 3' poly A tail if the sequence of interest lacks poly A.

Additionally, the vector may contain promoters or enhancers which increase gene expression. Such promoters are host specific and include but are not limited to MMTV, SV40, or metallothionine promoters for CHO cells; trp, lac, tac or T7 promoters for bacterial hosts; or alpha factor, alcohol oxidase or PGH promoters for yeast. Adenoviral vectors with or without transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, may be used to drive protein expression in mammalian cell lines. Once homogeneous cultures of recombinant cells are obtained, large quantities of recombinantly produced protein can be recovered from the conditioned medium and analyzed using chromatographic methods well known in the art. An alternative method for the production of large amounts of secreted protein involves the transformation of mammalian embryos and the recovery of the recombinant protein from milk produced by transgenic cows, goats, sheep, etc. Polypeptides and closely related molecules may be expressed recombinantly in such a way as to facilitate protein purification. One approach involves expression of a chimeric protein which includes one or more additional polypeptide domains not naturally present on human polypeptides.

Such purification-facilitating domains include, but are not limited to, metal-chelating peptides such as histidine-tryptophan domains that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase from Invitrogen (San Diego, CA) between the polypeptide sequence and the purification domain may be useful for recovering the polypeptide.

Immunoassays.

The polypeptides including their fragments or derivatives or analogs thereof of the present invention, or cells expressing them, can be in a variety of assays, many of which are described herein, for the detection of antibodies. They also can be used as an immunogen to produce antibodies. These antibodies can be, for example, polyclonal or monoclonal antibodies, chimeric, single chain and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

For example, antibodies generated against a polypeptide corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptide into an animal or by administering the polypeptide to an animal such as a mouse, rabbit, goat or human. A mouse, rabbit or goat is preferred. The antibody so obtained then will bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies that bind the native polypeptide. Such antibodies can then be used to isolate the polypeptide from test samples such as tissue suspected of containing that polypeptide. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique as described by Kohler and Milstein, Nature 256:495-497 (1975), the trioma technique, the human B-cell hybridoma technique as described by Kozbor et al, Immun. Today 4:72 (1983), and the EBV-hybridoma technique to produce human monoclonal antibodies as described by Cole, et al., in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc, New York, NY, pp. 77-96 (1985). Techniques described for the production of single chain antibodies can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. See, for example, U.S. Pat. No. 4,946,778, which is incorporated herein by reference.

Various assay formats may utilize the antibodies of the present invention, including "sandwich" immunoassays and probe assays. For example, the monoclonal antibodies or fragment thereof of the present invention can be employed in various assay systems to determine the presence, if any, of MMP19 polypeptide in a test sample. For example, in a first assay format, a polyclonal or monoclonal antibody or fragment thereof, or a combination of

these antibodies, which has been coated on a solid phase, is contacted with a test sample, to form a first mixture. This first mixture is incubated for a time and under conditions sufficient to form antigen/antibody complexes. Then, an indicator reagent comprising a monoclonal or a polyclonal antibody or a fragment thereof, or a combination of these antibodies, to which a signal generating compound has been attached, is contacted with the antigen/antibody complexes to form a second mixture. This second mixture then is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence of a MMP19 polypeptide antigen present in the test sample and captured on the solid phase, if any, is determined by detecting the measurable signal generated by the signal generating compound. The amount of MMP19 polypeptide antigen present in the test sample is proportional to the signal generated.

Or, a polyclonal or monoclonal MMP19 polypeptide antibody or fragment thereof, or a combination of these antibodies which is bound to a solid support, the test sample and an indicator reagent comprising a monoclonal or polyclonal antibody or fragments thereof, which specifically binds to MMP19 polypeptide antigen, or a combination of these antibodies to which a signal generating compound is attached, are contacted to form a mixture. This mixture is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence, if any, of MMP19 polypeptide present in the test sample and captured on the solid phase is determined by detecting the measurable signal generated by the signal generating compound. The amount of MMP19 polypeptide proteins present in the test sample is proportional to the signal generated.

In another assay format, one or a combination of at least two monoclonal antibodies of the invention can be employed as a competitive probe for the detection of antibodies to MMP19 polypeptide protein. For example, MMP19 polypeptide proteins such as the recombinant antigens disclosed herein, either alone or in combination, are coated on a solid phase. A test sample suspected of containing antibody to MMP19 polypeptide antigen then is incubated with an indicator reagent comprising a signal generating compound and at least one monoclonal antibody of the invention for a time and under conditions sufficient to form antigen/antibody complexes of either the test sample and indicator reagent bound to the solid phase or the indicator reagent bound to the solid phase. The reduction in binding of the monoclonal antibody to the solid phase can be quantitatively measured.

In yet another detection method, each of the monoclonal or polyclonal antibodies of the present invention can be employed in the detection of MMP19 polypeptide antigens in fixed tissue sections, as well as fixed cells by immunohistochemical analysis. Cytochemical analysis wherein these antibodies are labeled directly (with, for example, fluorescein, colloidal gold, horseradish peroxidase, alkaline phosphatase, etc.) or are labeled by using secondary

labeled anti-species antibodies (with various labels as exemplified herein) to track the histopathology of disease also are within the scope of the present invention.

In addition, these monoclonal antibodies can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific MMP19 polypeptide proteins from cell cultures or biological tissues such as to purify recombinant and native MMP19 polypeptide antigens and proteins.

The monoclonal antibodies of the invention can also be used for the generation of chimeric antibodies for therapeutic use, or other similar applications.

The monoclonal antibodies or fragments thereof can be provided individually to detect MMP19 polypeptide antigens. Combinations of the monoclonal antibodies (and fragments thereof) provided herein also may be used together as components in a mixture or "cocktail" of at least one MMP19 polypeptide antibody of the invention with antibodies to other MMP19 polypeptide regions, each having different binding specificities. Thus, this cocktail can include the monoclonal antibodies of the invention which are directed to MMP19 polypeptide proteins and other monoclonal antibodies to other antigenic determinants of the MMP19 polypeptide genome.

The polyclonal antibody or fragment thereof which can be used in the assay formats should specifically bind to a MMP19 polypeptide region or other MMP19 polypeptide proteins used in the assay. The polyclonal antibody used preferably is of mammalian origin; human, goat, rabbit or sheep anti- MMP19 polypeptide polyclonal antibody can be used. Most preferably, the polyclonal antibody is rabbit polyclonal anti-MMP19 polypeptide antibody. The polyclonal antibodies used in the assays can be used either alone or as a cocktail of polyclonal antibodies. Since the cocktails used in the assay formats are comprised of either monoclonal antibodies or polyclonal antibodies having different MMP19 polypeptide specificity, they would be useful for diagnosis, evaluation and prognosis of MMP19 polypeptide condition, as well as for studying MMP19 polypeptide protein differentiation and specificity.

It is contemplated and within the scope of the present invention that the MMP19 polypeptide may be detectable in assays by use of a recombinant antigen as well as by use of a synthetic peptide or purified peptide, which contains an amino acid sequence of MMP19 polypeptide. It also is within the scope of the present invention that different synthetic, recombinant or purified peptides identifying different epitopes of the MMP19 polypeptide can be used in combination in an assay to diagnose, evaluate, or prognose the disease condition. In this case, these peptides can be coated onto one solid phase, or each separate peptide may be coated on separate solid phases, such as microparticles, and then combined to form a mixture of peptides which can be later used in assays. Furthermore, it is contemplated that multiple peptides which define epitopes from different polypeptides may be used in combination to

make a diagnosis, evaluation, or prognosis of disease. Peptides coated on solid phases or labelled with detectable lables are then allowed to compete with peptides from a patient sample for a limited amount of antibody. A reduction in binding of the synthetic, recombinant, or purified peptides to the antibody (or antibodies) is an indication of the presence of MMP19 polypeptides in the patient sample which in turn indicates the presence of disease in the patient. Such variations of assay formats are known to those of ordinary skill in the art and are discussed herein below.

In another assay format, the presence of antibody and/or antigen to MMP19 polypeptide can be detected in a simultaneous assay, as follows. A test sample is simultaneously contacted with a capture reagent of a first analyte, wherein said capture reagent comprises a first binding member specific for a first analyte attached to a solid phase and a capture reagent for a second analyte, wherein said capture reagent comprises a first binding member for a second analyte attached to a second solid phase, to thereby form a mixture. This mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte and capture reagent/second analyte complexes. These so-formed complexes then are contacted with an indicator reagent comprising a member of a binding pair specific for the first analyte labeled with a signal generating compound and an indicator reagent comprising a member of a binding pair specific for the second analyte labeled with a signal generating compound to form a second mixture. This second mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte/indicator reagent complexes and capture reagent/second analyte/indicator reagent complexes. The presence of one or more analytes is determined by detecting a signal generated in connection with the complexes formed on either or both solid phases as an indication of the presence of one or more analytes in the test sample. In this assay format, recombinant antigens derived from human expression systems may be utilized as well as monoclonal antibodies produced from the proteins derived from the mammalian expression systems as disclosed herein. Such assay systems are described in greater detail in EP Publication No. 0473065.

In yet other assay formats, the polypeptides disclosed herein may be utilized to detect the presence of anti- MMP19 polypeptide in test samples. For example, a test sample is incubated with a solid phase to which at least one recombinant protein has been attached. These are reacted for a time and under conditions sufficient to form antigen/antibody complexes. Following incubation, the antigen/antibody complex is detected. Indicator reagents may be used to facilitate detection, depending upon the assay system chosen. In another assay format, a test sample is contacted with a solid phase to which a recombinant protein produced as described herein is attached and also is contacted with a monoclonal or polyclonal antibody specific for the protein, which preferably has been labeled with an indicator reagent. After incubation for a time and under conditions sufficient for

antibody/antigen complexes to form, the solid phase is separated from the free phase, and the label is detected in either the solid or free phase as an indication of the presence of MMP19 polypeptide antibody. Other assay formats utilizing the recombinant antigens disclosed herein are contemplated. These include contacting a test sample with a solid phase to which at least one antigen from a first source has been attached, incubating the solid phase and test sample for a time and under conditions sufficient to form antigen/antibody complexes, and then contacting the solid phase with a labeled antigen, which antigen is derived from a second source different from the first source. For example, a recombinant protein derived from a first source such as E. coli is used as a capture antigen on a solid phase, a test sample is added to the so-prepared solid phase, and a recombinant protein derived from a different source (i.e., non-E. coli) is utilized as a part of an indicator reagent. Likewise, combinations of a recombinant antigen on a solid phase and synthetic peptide in the indicator phase also are possible. Any assay format which utilizes an antigen specific for MMP19 polypeptide from a first source as the capture antigen and an antigen specific for MMP19 polypeptide from a different second source are contemplated. Thus, various combinations of recombinant antigens, as well as the use of synthetic peptides, purified proteins, and the like, are within the scope of this invention. Assays such as this and others are described in U.S. Patent No. 5,254,458, which enjoys common ownership and is incorporated herein by reference.

Other embodiments which utilize various other solid phases also are contemplated and are within the scope of this invention. For example, ion capture procedures for immobilizing an immobilizable reaction complex with a negatively charged polymer (described in EP publication 0326100 and EP publication No. 0406473), can be employed according to the present invention to effect a fast solution-phase immunochemical reaction. An immobilizable immune complex is separated from the rest of the reaction mixture by ionic interactions between the negatively charged poly-anion/immune complex and the previously treated, positively charged porous matrix and detected by using various signal generating systems previously described, including those described in chemiluminescent signal measurements as described in EPO Publication No. 0 273,115.

Also, the methods of the present invention can be adapted for use in systems which utilize microparticle technology including in automated and semi-automated systems wherein the solid phase comprises a microparticle (magnetic or non-magnetic). Such systems include those described in published EPO applications Nos. EP 0 425 633 and EP 0 424 634, respectively.

The use of scanning probe microscopy (SPM) for immunoassays also is a technology to which the monoclonal antibodies of the present invention are easily adaptable. In scanning probe microscopy, in particular in atomic force microscopy, the capture phase, for example, at least one of the monoclonal antibodies of the invention, is adhered to a solid phase and a

scanning probe microscope is utilized to detect antigen/antibody complexes which may be present on the surface of the solid phase. The use of scanning tunneling microscopy eliminates the need for labels which normally must be utilized in many immunoassay systems to detect antigen/antibody complexes. The use of SPM to monitor specific binding reactions can occur in many ways. In one embodiment, one member of a specific binding partner (analyte specific substance which is the monoclonal antibody of the invention) is attached to a surface suitable for scanning. The attachment of the analyte specific substance may be by adsorption to a test piece which comprises a solid phase of a plastic or metal surface, following methods known to those of ordinary skill in the art. Or, covalent attachment of a specific binding partner (analyte specific substance) to a test piece which test piece comprises a solid phase of derivatized plastic, metal, silicon, or glass may be utilized. Covalent attachment methods are known to those skilled in the art and include a variety of means to irreversibly link specific binding partners to the test piece. If the test piece is silicon or glass, the surface must be activated prior to attaching the specific binding partner. Also, polyelectrolyte interactions may be used to immobilize a specific binding partner on a surface of a test piece by using techniques and chemistries. The preferred method of attachment is by covalent means. Following attachment of a specific binding member, the surface may be further treated with materials such as serum, proteins, or other blocking agents to minimize non-specific binding. The surface also may be scanned either at the site of manufacture or point of use to verify its suitability for assay purposes. The scanning process is not anticipated to alter the specific binding properties of the test piece.

While the present invention discloses the preference for the use of solid phases, it is contemplated that the reagents such as antibodies, proteins and peptides of the present invention can be utilized in non-solid phase assay systems. These assay systems are known to those skilled in the art, and are considered to be within the scope of the present invention.

It is contemplated that the reagent employed for the assay can be provided in the form of a test kit with one or more containers such as vials or bottles, with each container containing a separate reagent such as a probe, primer, monoclonal antibody or a cocktail of monoclonal antibodies, or a polypeptide (either recombinant or synthetic) employed in the assay. Other components such as buffers, controls, and the like, known to those of ordinary skill in art, may be included in such test kits. It also is contemplated to provide test kits which have means for collecting test samples comprising accessible body fluids, eg. blood, urine, saliva, and stool. Such collection means include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; cups for collecting and stabilizing urine or stool samples. Collection materials, papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. The collection materials also may be treated with or contain preservatives, stabilizers or

antimicrobial agents to help maintain the integrity of the specimens. Test kits designed for the collection, stabilization, and preservation of test specimens obtained by surgery or needle biopsy are also useful. It is contemplated that all kits may be configured in two components; one component for collection and transport of the specimen, and the other component for the analysis of the specimen. Further, kits for the collection, stabilization, and preservation of test specimens may be configured for use by untrained personnel and may be available in the open market for use at home with subsequent transportation to a laboratory for analysis of the test sample.

E. coli bacteria (clone #907334) has been deposited at the American Type Culture Collection (A.T.C.C.), 12301 Parklawn Drive, Rockville, Maryland 20852, as of March 10, 1997, under the terms of the Budapest Treaty and will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit, or for the enforceable period of the U.S. patent, whichever is longer. The deposit and any other deposited material described herein are provided for convenience only, and are not required to practice the present invention in view of the teachings provided herein. The cDNA sequence in all of the deposited material is incorporated herein by reference. Clone #907334 was accorded A.T.C.C. Deposit No _____.

Having now generally described the invention, a complete understanding can be obtained by reference to the following specific examples. The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

Example 1: Isolation of Human cDNA Clones Homologous to MMPs

A consensus of twelve human matrix metalloprotease amino acid sequences was obtained by aligning sequences using the PILEUP program in the Wisconsin Sequence Analysis Package (Genetics Computer Group [GCG], Madison, WI). The consensus sequence was derived from a plurality of seven sequences at a given amino acid position. The letter "x" was substituted for each amino acid that differed from the determined consensus residue and was used to represent any amino acid.

The MMP consensus sequence (SEQ ID NO:7, see FIG. 1) was used to search the LifeSeq™ human expression database (Incyte Pharmaceuticals, Inc., Palo Alto, CA) for human MMP sequences. This database is comprised of partial sequences of cDNA clone inserts, so-called expressed sequence tags (ESTs), derived from cDNA libraries from various human tissues. A search of LIFESEQ™ using the BLAST and Smith-Waterman similarity search algorithms with the MMP consensus sequence identified an EST (from clone #907334) unique to the LIFESEQ™ database and whose deduced amino acid sequence of one reading

frame contained the amino acid sequence PRCGVTD (SEQ ID NO:8), similar to the "cysteine switch" motif in the propeptide region of all MMPs. The EST from clone #907334 was derived from a colon tissue library using oligo-dT for the reverse transcription reaction. The cDNA clone #907334 in pSPORT1 plasmid (GIBCO BRL, Gaithersburg, MD) was obtained for further study.

Clone #907334 was amplified in bacteria and digested with the restriction enzymes EcoRI and NotI to determine the size of the cDNA insert. Insert length was determined to be approximately 1900 base pairs. The complete nucleotide sequence of the clone (shown in FIG. 4) was obtained by initially sequencing with plasmid primers and subsequently with primers near the end of each newly obtained sequence. All sequence information from each reaction was compiled and analyzed using the Sequencher™ program (Gene Codes Corporation, Ann Arbor, MI). Sequencing was continued until both strands of the cloned cDNA insert were sequenced in entirety. In FIG. 4, the sense strand (SEQ ID NO:1) is shown on top with its complement (SEQ ID NO:2) aligned directly beneath.

The deduced amino acid sequence of clone #907334 (SEQ ID NO:10, also shown in FIG. 4) encoded a polypeptide of at least 470 amino acids but lacked an initiating methionine start codon and a portion of the putative pro-peptide domain. The amino acid sequence was compared to those of other MMPs (FIG. 5) and shown to contain a cysteine switch motif (SEQ ID NO:8) within the putative pro-peptide domain which was 86% homologous to the consensus cysteine switch motif of the other MMPs (PRCGVPD, SEQ ID NO:11). In addition, MMP19 contained an identical version (100%) of the highly conserved zinc binding consensus sequence (HEXaaGHXaaLGLXaaHS, SEQ ID NO:12) found in the putative catalytic domain. The sequence also contained a potential recognition and cleavage site (RXKR, SEQ ID NO:33) for Kex2-like proteases shown to be involved in enzyme activation of stromelysin 3 and the membrane-type MMPs.

Example 2: Localization of Clone #907334 Transcripts

A. Nothern Blotting: The entire nucleotide sequence of clone #907334 (hereafter referred to as MMP19) was compared to LIFESEQ™ and other databases (for example GenBank, Wash. U./Merck EST) using the BLAST and Smith-Waterman programs. Public databases did not contain sequences identical to MMP19 cDNA. LIFESEQ™ contained eight additional ESTs identical to some portion of MMP19 cDNA (FIG. 6). The nine ESTs in LIFESEQ™ which were identical to MMP19 DNA had been sequenced from cDNA libraries derived from 8 different tissues, including colon, lung, prostate, spinal cord, ovary and prostate tumor, brain tumor and ganglioneuroma (FIG. 6) Thus, these tissues expressed at least small amounts of the mRNA encoded by MMP19 cDNA.

In order to determine whether MMP19 mRNA sequences could be detected by hybridization, the entire 1717 bp fragment of MMP19 cDNA was radiolabelled with ^{32}P using a commercial random primer labeling kit (Pharmacia, Piscataway, NJ). Specific activity of the labeled fragment was determined to be ~5 x 10^8 cpm/μg DNA. The labeled cDNA was used as a probe to hybridize to mRNA (2 μg polyA+ RNA) on Northern blots (specifically, Multiple Tissue Northerns 1 and 2, obtained from Clontech, Palo Alto, CA) which contained various human tissue RNA. The blots were prehybridized at 60°C for 1 hour in Express Hyb solution (supplied with the blots) and hybridized (also in Express Hyb solution) at the same temperature for two hours in the presence of denatured probe at 1 x 10^6 cpm/mL. After washing the blots once in 2X SSPE + 0.1% SDS (20 min), and twice under stringent conditions (0.2X SSPE + 0.1% SDS, 50°C, 20 min. each wash), the filters were exposed and analyzed on a Phosphorimager (Molecular Dynamics, Sunnyvale, CA). By Northern blotting, it was determined that MMP19 mRNA transcript is approximately 2.4 kb and is present in human testes and to a lesser extent in human colon, ovary, small intestine, and prostate tissue (FIG. 11). Smaller less abundant transcripts with similar tissue distribution were also observed.

B. Ribonuclease Protection Assay: Alternatively, instead of or in addition to performing a Northern blot, a ribonuclease protection assay may be performed to determine whether MMP19 is present in particular tissues. A ribonuclease protection assay is performed as follows:

1. Labeling of Complementary RNA (cRNA) Hybridization Probes. Labeled sense and antisense riboprobes are transcribed from the EST sequence which contains an RNA polymerase promoter such as SP6 or T7. The sequence may be from a vector containing the appropriate EST insert or from a PCR-generated product of the insert using PCR primers which incorporate am RNA polymerase promoter sequence. The transcripts are prepared in a 20 μL reaction volume containing 1 μg of DNA template, 2 μL of 100 mM dithiothreitol, 0.8 μL of RNasin (10-40U), 500 μM each of ATP, CTP, GTP, 5 μL (alpha^{32}P) UTP or 100-500 μM biotinylated UTP, and 1 μL of RNA polymerase in transcription buffer (40 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 2 mM spermidine HCl, 5 mM NaCl). Following incubation at 37∞C for one hour, the transcripts are treated with DNase I (15 U) for an additional 30 min to digest the template. The probes then are isolated by spin columns, salt precipitation or electrophoresis techniques which are well-known in the art. Finally, the probes are dissolved in lysis buffer (5 M Guanidine Thiocyanate, 0.1 M EDTA, pH 7.0).

2. Hybridization of Labeled Probe to Target. Approximately 20 μg of extracted total cellular RNA, prepared as described in Sambrook, *et al. supra*, is placed in 10 μL of lysis buffer and mixed with either (i) 1 x10^5 cpm of radioactively labeled probe or (ii) 250 pg of non-isotopically labeled probe, each in 2 μL of lysis buffer. The mixture then is

incubated at 60∞C for 5 min and hybridized overnight at room temperature. See, T. Kaabache *et al.*, *Anal. Biochem.* **232:** 225-230 (1995).

3. RNase Digestion. Hybridizations are terminated by incubation with 380 µL of a solution containing 40 µg/mL RNase A and 625 U/mL RNase T1 in 1 mM EDTA, 300 mM NaCl, 30 mM Tris-HCl pH 7.4 for 45-60 min at room temperature. RNase digestion then is terminated by the addition of 60 µL of proteinase-K (1.7 mg/mL) containing 3.3% SDS, followed by incubation for 30 min at 37°C. The digested mixture then is extracted with phenol:chloroform:isoamyl alcohol to remove protein. The mRNA:cRNA hybrids are precipitated from the aqueous phase by the addition 4 µg yeast tRNA and 800 µL of ethanol, and incubation at -80°C for 30 min. The precipitates are collected by centrifugation.

4. Fragment Analysis. The precipitates are dissolved in 5 µL of denaturing gel loading dye (80% formamide, 10 mM EDTA, pH 8.0, 1 mg/mL xylene cyanol, 1 mg/mL bromophenol blue) and electrophoresed in 6 % polyacrylamide TBE, 8 M urea denaturing gels. The gels are dried under vacuum and autoradiographed. Quantitation can be performed by comparing the counts obtained from the test samples to a calibration curve that was generated by utilizing calibrators that are the sense strand. In cases where non-isotopic labels are used, hybrids are transferred from the gels to membranes (nylon or nitrocellulose) by blotting and then analyzed using detection systems that employ streptavidin alkaline phosphatase conjugates and chemiluminesence or chemifluoresence reagents. Again, expression of an mRNA which is detectable by the labeled probe in a particular tissue suggests that MMP19 is produced in that tissue.

C. Dot Blot/Slot Blot: Dot and slot blot assays are also quick methods to evaluate the presence of a specific nucleic acid sequence in a complex mix of nucleic acid. To perform, up to 20 µg of RNA is mixed in 50 µL of 50% formamide, 7% formaldehyde, 1X SSC, incubate 15 min at 68°C and cool on ice. Then, 100 µL of 20X SSC is added to the RNA mixture and loaded under vacuum onto a manifold apparatus that has a prepared nitrocellulose or nylon membrane. The membrane is soaked in water, 20X SSC for 1 hour, placed on two sheets of 20X SSC prewet Whatman #3 filter paper, and loaded into a slot blot or dot blot vacuum manifold apparatus. The slot blot is analyzed with probes prepared and labeled as described *supra*. Other methods and buffers not specifically detailed here are described in J. Sambrook *et al.*, *supra*.

D. In Situ Hybridization: This method is useful to directly detect specific target nucleic acid sequences in cells using detectable nucleic acid hybridization probes.

Tissues are prepared with cross-linking fixatives agents such as paraformaldehyde or glutaraldehyde for maximum cellular RNA retention. See, L. Angerer *et al.*, *Methods in Cell Biol.* **35:** 37-71 (1991). Briefly, the tissue is placed in greater than 5 volumes of 1% glutaraldehyde in 50 mM sodium phosphate, pH 7.5 at 4°C for 30 min. The solution is

changed with fresh solution for a further 30 min fixing. The fixing solution should have an osmolality of approximately 0.375% NaCl. The tissue is washed once in isotonic NaCl to remove the phosphate.

The fixed tissues then are embedded in paraffin, as follows. The tissue is dehydrated through a series of ethanol concentrations for 15 min each: 50% twice, 70% twice, 85%, 90% and 100% twice. The tissue next is soaked in two changes of xylene for 20 min each at room temperature; then it is soaked in two changes of 1 xylene:1 paraffin for 20 min each at 60°C; and then it is soaked in three final changes in paraffin for 15 min each.

The tissue next is cut in 5 μm sections using a standard microtome and placed on a slide previously treated with the tissue adhesive 3-aminopropyltriethoxysilane.

Paraffin is removed from the tissue by two 10 min xylene soaks and rehydrated in a series of ethanol concentrations; 99% twice, 95%, 85%, 70%, 50%, 30% and distilled water twice. The sections are pre-treated with 0.2 M HCl for 10 min and permeabilized with 2 μg/mL Proteinase-K at 37°C for 15 min.

Labeled riboprobes transcribed from the pSPORT1 plasmid containing fragments of MMP19 cDNA are hybridized to the prepared tissue sections and hybridized overnight at 56∞C in 3X standard saline extract and 50% formamide. Excess probe is removed by washing in 2X standard saline citrate and 50% formamide followed by digestion with 100 μg/mL RNase A at 37∞C for 30 min. Fluorescence probe is visualized by illumination with UV light under a microscope. Fluorescence in the cytoplasm is indicative of mRNA production. Fluorescence in the nucleus detects the presence of genomic material. Alternatively, the sections can be visualized by autoradiography.

Example 3: Expression of MMP19 Sequences

A. Construction of Expression Vectors Containing DNA Fragments Encoding the Catalytic Domains of MMP19 Protein: The MMP19 plasmid was used as template in PCR reactions to generate DNA fragments encoding MMP19 protein for introduction into two eukaryotic expression vectors, pcDNA3.1 (Invitrogen, San Diego, CA) and pCINeo (Promega, Madison, WI). The two upstream primers used in PCR reactions, SEQ ID NO:3 (5'-GTATCTCTAGACACCATGTTTGCAAAGCAAGGTAACAAATGGTACAAGC-3') and SEQ ID NO:4 (5'-GTATCTCTAGACACCATGAAGGTTCTGTGGGCTGCGTTGCTGGT-CACATTCCTGGCAGGATGCCAGGCCTTTGCAAAGCAAGGTAACAAATGG-3') contained sequences to create an XbaI restriction enzyme site, a Kozak consensus sequence for translation initiation, an ATG start codon, and a sequence of nucleotides from nucleotide position 214 to nucleotide position 244 of SEQ ID NO:1, which corresponded to the N-terminus of the putative catalytic domain of the enzyme. SEQ ID NO:4 also contained a

sequence which encodes a signal peptide (from the secreted protein, apolipoprotein E), for transport of the translated protein into the secretory pathway. The signal peptide sequence was placed upstream from and in frame with the sequence encoding the N-terminus of the putative catalytic domain. Downstream primers used in PCR reactions were either SEQ ID NO:5 (5'-GTACTTCTAGACTACTTGTCAT-CGTCGTCCTTGTAGTCACCACCGAACAGGGCG-CTCCCCGAGTTGGCATGCC-3') which encodes an epitope recognized by anti-FLAG M2, (available from Sigma Biosciences, St. Louis, MO) or SEQ ID NO:6 (5'-GTACTTCTAGA-GATCTTCTTCACTGATCAGCTTCTGTTCACCACCGAACAGGGCGCTCCCCGAGTTG-GCATGCC-3') which encodes an epitope recognized by anti-c-Myc (Santa Cruz Biotechnology, Santa Cruz, CA). In both downstream primers, these epitope sequences were in frame with the last codon in the open reading frame of the cDNA and were followed by a stop codon (TGA) and an XbaI restriction enzyme site.

PCR amplifications were performed using primers sets SEQ ID NOS:3 and 5, SEQ ID NOS:3 and 6, SEQ ID NOS:4 and 5 and SEQ ID NOS:4 and 6 under standard PCR conditions, i.e. in a total reaction volume of 50 µL containing 200 µM of each dNTP wherein N was A, T, G and C, 1 µM of each primer, ~50 ng template DNA and 1 unit of *AmpliTaq* DNA polymerase (Perkin Elmer, Norwalk, CT). Amplifications were performed for a total of 35 cycles (1 cycle = 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min). After amplification, PCR products were digested with XbaI, gel purified, and ligated into the XbaI site of pCDNA3.1 and pCINeo. After transformation of the ligated DNA into appropriate bacterial hosts (for example DH5a), plasmid DNA was prepared from individual clones and subjected to restriction enzyme and sequence analysis to identify clones that contained MMP19 DNA with the correct sequence and in the proper orientation.

B. Transfection of MMP19 DNA Expression Vectors into HEK293 Cells: Expression of MMP19 constructs in HEK293 cells is achieved by transfection using a modified calcium-phosphate procedure (Chen and Okayama, *Mol. Cell. Biol.* 7: 2745-2752, 1987). HEK-293 cells (ATCC CRL1573) are grown in minimal essential medium (MEM) containing 10% fetal bovine serum (FBS) and antibiotic/antimycotic solution at 37° C in 5% CO_2, 95% O_2. Cells are transfected with purified plasmid MMP19 DNA (20 µg/2.5 X 10^6 cells/10 cm plate). For stable transfections, cells are grown in the presence of G418 and MEM/FBS. For both transient and stable transfections, serum-free media is harvested 3 days after medium replacement.

C. Detection of MMP19 protein (Western blot): Media from transfected cells is mixed (vol:vol, 1:1) with 2X denaturing buffer (10 mM Tris-HCl, pH6.8, 4% SDS, 20% glycerol, 1% ß-mercaptoethanol, 0.02% bromphenol blue) and denatured by heating 5 minutes, 95°C. Aliquots (20µl) of each sample are electrophoresed for ~1 hour on a 10-20% Tris-tricine minigel (10 X 10 cm) at ~50 mA constant current. Gels are transferred to PVDF membranes

by electroblotting at 200 mA for 1.5 hours. Non-specific binding sites on PVDF membranes are blocked for 1 hour at room temperature with 5% nonfat dry milk in Tris-buffered saline containing Tween-20 (TBS-Tween) (20 mM Tris, pH 7.6, 140 mM NaCl, 0.1% Tween-20). Blots are then incubated for 1 hour at room temperature in TBS-Tween/5% milk containing an appropriate amount of primary antibody (either anti-FLAG or anti-c-Myc). Blots are washed in 3 changes of TBS-Tween for a total of 45 minutes at room temperature before incubating with the detection antibody (1:1000 dilution) for 1 hour at room temperature in TBS-Tween/5% milk. Blots are again washed in 3 changes of TBS-Tween for 45 minutes at room temperature. Protein bands are visualized by autoradiography using ECL Western blotting detection reagents (Amersham, Arlington Heights, IL).

Example 4: Assays of Enzymatic Activity of MMP19

Purification of the MMP19 protein containing the FLAG sequence is performed by immunoaffinity chromatography using an affinity matrix comprising anti-FLAG M2 monoclonal antibody covalently attached to agarose by hydrazide linkage (Eastman Kodak Co., New Haven, CT). Prior to affinity purification, medium from transfected HEK293 cells is exchanged into 50 mM Tris-HCl pH 7.5, 150 mM NaCl buffer using a Sephadex G-25 (Pharmacia Biotech Inc., Uppsala, Sweden) column. Protein in this buffer is applied to the anti-FLAG M2 antibody affinity column, non-binding protein is eluted by washing the column with 50 mM Tris-HCl pH 7.5, 150 mM NaCl buffer, and bound protein is eluted using an excess of FLAG peptide in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl. The excess FLAG peptide is removed by size exclusion chromatography and column fractions containing MMP19 protein are dialyzed in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 MM CaCl.

Assays to determine enzyme activity are performed using a fluorogenic peptide substrate Gly-Glu(EDANS)-Gly-Pro-Leu-Gly-Leu-Tyr-Ala-Lys(DABCYL)-Gly (SEQ ID NO:13). SEQ ID NO:13 was synthesized and purified acording the the procedure of E.D. Matayoshi *et al.*, *Science*, 247: 954-958, 1990). Hydrolysis of the Gly-Leu bond in SEQ ID NO:13 results in a 40-fold increase in fluorescence.

Purified MMP19 protein from transfected HEK293 cell medium is serially diluted and incubated in microtiter plate wells with 100 μM fluorogenic peptide substrate, 50 mM Tris-HCl, pH 7.5, 150 mM NaCl in a final volume of 150 μL. Assay mixtures are incubated at room temperature and the progress of the reaction monitored for up to 1 hour in an Titertek Fluoroskan II instrument (ICN Biomedicals, Huntsville, AL) with an excitation filter set at 335 nm and emission filter at 485 nm. Background fluorescence is determined using identical reactions in the absence of MMP19. Data is collected online with a Macintosh computer using DELTA SOFT II, version 4.0 (BioMetallics, Inc., Princeton, NJ). Nonlinear curve fitting is

performed using KaleidaGraph (Synergy Software, Reading, PA). Compounds which potentially inhibit MMP19 activity are screened by including serial dilutions of each compound with the above reaction and comparing the MMP19 enzymatic activity in the presence and absence of compound.

Example 5: Proteolysis of pro-MMPs by MMP19

Interaction of MMP19 with other MMPs is determined by incubation of purified active MMP19 with the zymogen (pro-peptide) forms of Gelatinase-A (72 kD) [MMP-2] and Gelatinase-B (92 kD) [MMP-9]. Gelatinase-A and Gelatinase-B are isolated from HT-1080 cell cultures stimulated with TNFa as described in *J. Antibiotics* 45: 1733-1737, 1992 and *Biochem. J.* 285: 603-611, 1992. After 1 hour incubation at 37°C in buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl and approximately 250 ng of each enzyme, aliquots are removed and electrophoresed on 10-20% Tris-Tricine gels. Western blots of gels are performed using antibodies to Gelatinse A and Gelatinase B (Oncogene Science, Cambridge, MA) as described above and proteolysis is indicated by the presence of bands of lower molecular weight relative to the pro-enzyme forms of Gelatinase A and Gelatinase B.

Example 6: Production of Polyclonal Antibodies to MMP19

Synthetic peptides (SEQUENCE ID NOS:14-17, see Table 1 below) are prepared based upon the predicted amino acid sequence of the MMP19 polypeptide.

Table 1

Sequence	SEQ ID NO:
RHRTKMRRKKRFAKQGN	14
FQGDHNDGLGNAFDG	15
RSLQDWGGIPEEVSGALPRPDGSII	16
ATELPWMGCWHANSGSALF	17

Peptides are synthesized on an ABI Peptide Synthesizer (Applied Biosystems, Foster City, CA), using standard reagents and conditions known in the art for solid phase peptide synthesis (see for example, Stewart, J.M., and Young, D.J., *Solid Phase Peptide Synthesis*, W.H. Freeman Co., San Francisco, 1963). Cleavage of the peptide from the resin and final deprotection of the peptide are achieved by adding the resin to 20 ml trifluoroacetic acid (TFA), 0.3 ml water, 0.2 ml ethanedithiol, 0.2 ml thioanisole and 100 mg phenol, and stirring at room temperature for 1.5 hours. The resin then is filtered by suction and the peptide obtained by

precipitation of the TFA solution with ether, followed by filtration. Each peptide is purified via reverse-phase preparative HPLC using a water/acetonitrile/0.1% TFA gradient and lyophilized. The product is confirmed by mass spectrometry.

To generate antigens for immunization, the purified peptides are conjugated to Keyhole Limpet Hemocyanin (KLH) and bovine serum albumin (BSA) using an Imject Activated Immunogen Conjugation Kit (Pierce, Rockford, IL) in accordance with the manufacturer's instructions.

Polyclonal antisera are generated using the protocol of the Berkeley Antibody Company (Berkeley, CA). Before receiving the first immunization, a sample of preimmune blood (5 ml) is drawn from each of at least 2 rabbits. Afterward, each rabbit is injected subcutaneously with an aliquot of KLH-conjugated peptide (200-500 mg) in Complete Freunds Adjuvant. After 21 days, the immune response is boosted with a second subcutaneous injection of KLH-conjugated peptide (100-250 mg) in Incomplete Freund's Adjuvant. Blood (50 ml) is collected on day 31 and serum tested for reactivity to BSA-coupled peptide using an enzyme linked immunoadsorbant assay (ELISA). Subsequent boosts with KLH-conjugated peptide are given on days 42, 63 and 84 (post injection #1) and production bleeds (50 ml) drawn on days 52, 73 and 94 for testing by ELISA to determine antibody titer. Serum is then stored at -20°C until further use.

Example 7: Isolation of the 5' end of MMP19 cDNA

CapFinder cDNA synthesis kit (Clontech, Palo Alto, CA) was used to amplify DNA at the 5' end of MMP19 cDNA. Briefly, reverse transcription reactions using human testes mRNA (Clontech, Palo Alto, CA) were performed as specified by the manufacturer. The cDNA was amplified by PCR using an oligonucleotide primer complementary to MMP19 cDNA (nucleotides 704-678 of SEQ ID NO:1) and the 5' CapSwitch oligonucleotide. PCR amplifications were performed in a total reaction volume of 100 µL containing 200 µM of each dNTP wherein N was A, T, G and C, 1 µM of each primer, 20 µL of cDNA from the reverse transcription reaction, and 1 unit of *AmpliTaq* DNA polymerase (Perkin Elmer, Norwalk, CT). Amplifications were performed for a total of 35 cycles (1 cycle = 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min). Two PCR reaction products (1.3 kb and 1.0 kb) identified by agarose gel electrophoresis and ethidium bromide staining were large enough to contain sequences upstream of the 5' end of the original sequence. These fragments were purified and subcloned into pCR-Script (Stratagene, La Jolla, CA) in accordance with the protocol provided by the supplier.

Clones are analyzed for sequences encoding the putative 5' end of MMP19 mRNA using an oligonucleotide complementary to MMP19 cDNA (nucleotides 704-678 of SEQ ID NO:1) in standard nucleotide sequencing reactions. Clones are verified as authentic MMP19

cDNA by PCR amplification of predicted size bands using testes mRNA as template and primers within both the new sequence and complementary to the original MMP19 cDNA sequence (nucleotides 655-626 of SEQ ID NO:1).

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Falduto, Michael
Magnuson, Scott R.
Morgan, Douglas W. Morgan

(ii) TITLE OF THE INVENTION: HUMAN MATRIX METALLOPROTEASE, PROTEINS ENCODED THEREFROM AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 33

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Abbott Laboratories
(B) STREET: 100 Abbott Park Road
(C) CITY: Abbott Park
(D) STATE: IL
(E) COUNTRY: USA
(F) ZIP: 60064-3500

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 11-MAR-1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Casuto, Dianne
(B) REGISTRATION NUMBER: P-40,943
(C) REFERENCE/DOCKET NUMBER: 6073.US.01

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (847) 938-3137
(B) TELEFAX: (847) 938-2623
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1717 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAAGCTCCCA CCTCCACTCG ATTCAGCGAT GCCATCAGAG CGTTTCAGTG GGTGTCCCAG 60
CTACCTGTCA GCGGCGTGTT GGACCGCGCC AACCTGCGCC AGATGACTCG TCCCCGCTGC 120
GGGGTTACAG ATACCAACAG TTATGCGGCC TGGGCTGAGA GGATCAGTGA CTTGTTTGCT 180
AGACACCGGA CCAAAATGAG GCGTAAGAAA CGCTTTGCAA AGCAAGGTAA CAAATGGTAC 240
AAGCAGCACC TCTCCTACCG CCTGGTGAAC TGGCCTGAGC ATCTGCCGGA GCCGGCAGTT 300
CGGGGCGCCG TGCGCGCCGC CTTCCAGTTG TGGAGCAACG TCTCAGCGCT GGAGTTCTGG 360
GAGGCCCCAG CCACAGGCCC CGCTGACATC CGGCTCACCT TCTTCCAAGG GGACCACAAC 420
GATGGGCTGG GCAATGCCTT TGATGGCCCA GGGGGCGCCC TGGCGCACGC CTTCCTGCCC 480
CGCCGCGGCG AAGCGCACTT CGACCAAGAT GAGCGCTGGT CCCTGAGCCG CCGCCGCGGG 540
CGCAACCTGT TCGTGGTGCT GGCGCACGAG ATCGGTCACA CGCTTGGCCT CACCCACTCG 600
CCCGCGCCGC GCGCGCTCAT GGCGCCCTAC TACAAGAGGC TGGGCCGCGA CGCGCTGCTC 660
AGCTGGGACG ACGTGCTGGC CGTGCAGAGC CTGTATGGGA AGCCCCTAGG GGGCTCAGTG 720
GCCGTCCAGC TCCCAGGAAA GCTGTTCACT GACTTTGAGA CCTGGGACTC CTACAGCCCC 780
CAAGGAAGGC GCCCTGAAAC GCAGGGCCCT AAATACTGCC ACTCTTCCTT CGATGCCATC 840
ACTGTAGACA GGCAACAGCA ACTGTACATT TTTAAAGGGA GCCATTTCTG GGAGGTGGCA 900
GCTGATGGCA ACGTCTCAGA GCCCCGTCCA CTGCAGGAAA GATGGGTCGG GCTGCCCCCC 960
AACATTGAGG CTGCGGCAGT GTCATTGAAT GATGGAGATT TCTACTTCTT CAAAGGGGGT 1020
CGATGCTGGA GGTTCCGGGG CCCCAAGCCA GTGTGGGGTC TCCCACAGCT GTGCCGGGCA 1080
GGGGGCCTGC CCCGCCATCC TGACGCCGCC CTCTTCTTCC CTCCTCTGCG CCGCCTCATC 1140
CTCTTCAAGG GTGCCCGCTA CTACGTGCTG GCCCGAGGGG GACTGCAAGT GGAGCCCTAC 1200
TACCCCCGAA GTCTGCAGGA CTGGGGAGGC ATCCCTGAGG AGGTCAGCGG CGCCCTGCCG 1260
AGGCCCGATG GCTCCATCAT CTTCTTCCGA GATGACCGCT ACTGGCGCCT CGACCAGGCC 1320
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CATGCCAACT CGGGGAGCGC CCTGTTCTGA AGGCACCTCC TCACCTCAGA AACTGGTGGT 1440
GCTCTCAGGG CAAAATCATG TTCCCCACCC CCGGGGCAGA ACCCCTCTTA GAAGCCTCTG 1500
AGTCCCTCTG CAGAAGACCG GGCAGCAAAG CCTCCATCTG GAAGTCTGTC TGCCTTTGTT 1560
CCTTGAAGAA TGCAGCATTG TCTTTGTCTG TCCCCACCAC ATGGAGGTGG GGGTGGGATC 1620
AATCTTAGGA AAAGCAAAAA AGGGTCCCAG ATCCCTTGGC CCTTTCCTCC GAAAAAAAAA 1680
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAA 1717

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1717 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTTCGAGGGT GGAGGTGAGC TAAGTCGCTA CGGTAGTCTC GCAAAGTCAC CCACAGGGTC 60
GATGGACAGT CGCCGCACAA CCTGGCGCGG TTGGACGCGG TCTACTGAGC AGGGGCGACG 120
CCCCAATGTC TATGGTTGTC AATACGCCGG ACCCGACTCT CCTAGTCACT GAACAAACGA 180

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TCTGTGGCCT GGTTTTACTC CGCATTCTTT GCGAAACGTT TCGTTCCATT GTTTACCATG    240
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GCCCCGCGGC ACGCGCGGCG GAAGGTCAAC ACCTCGTTGC AGAGTCGCGA CCTCAAGACC    360
CTCCGGGGTC GGTGTCCGGG GCGACTGTAG GCCGAGTGGA AGAAGGTTCC CCTGGTGTTG    420
CTACCCGACC CGTTACGGAA ACTACCGGGT CCCCCGCGGG ACCGCGTGCG GAAGGACGGG    480
GCGGCGCCGC TTCGCGTGAA GCTGGTTCTA CTCGCGACCA GGGACTCGGC GGCGGCGCCC    540
GCGTTGGACA AGCACCACGA CCGCGTGCTC TAGCCAGTGT GCGAACCGGA GTGGGTGAGC    600
GGGCGCGGCG CGCGCGAGTA CCGCGGGATG ATGTTCTCCG ACCCGGCGCT GCGCGACGAG    660
TCGACCCTGC TGCACGACCG GCACGTCTCG GACATACCCT TCGGGGATCC CCCGAGTCAC    720
CGGCAGGTCG AGGGTCCTTT CGACAAGTGA CTGAAACTCT GGACCCTGAG GATGTCGGGG    780
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CGACTACCGT TGCAGAGTCT CGGGGCAGGT GACGTCCTTT CTACCCAGCC CGACGGGGGG    960
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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GTATCTCTAG ACACCATGAA GGTTCTGTGG GCTGCGTTGC TGGTCACATT CCTGGCAGGA    60
TGCCAGGCCT TTGCAAAGCA AGGTAACAAA TGG                                 93
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTACTTCTAG ACTACTTGTC ATCGTCGTCC TTGTAGTCAC CACCGAACAG GGCGCTCCCC 60
GAGTTGGCAT GCC 73

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 74 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTACTTCTAG AGATCTTCTT CACTGATCAG CTTCTGTTCA CCACCGAACA GGGCGCTCCC 60
CGAGTTGGCA TGCC 74

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 820 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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50 55 60
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65 70 75 80
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85 90 95
Gly Lys Leu Asp Xaa Xaa Thr Leu Glu Xaa Met Xaa Lys Pro Arg Cys

100 105 110
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115 120 125
Phe Xaa Leu Xaa Pro Xaa Xaa Pro Lys Trp Xaa Xaa Xaa Xaa Xaa Thr
130 135 140
Tyr Arg Ile Xaa Asn Tyr Thr Pro Asp Leu Xaa Xaa Xaa Xaa Val Asp
145 150 155 160
Xaa Ala Ile Xaa Lys Ala Phe Xaa Val Trp Ser Xaa Val Thr Pro Leu
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Xaa Phe Xaa Xaa Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Glu Gly
180 185 190
Xaa Ala Asp Ile Met Ile Xaa Phe Ala Xaa Xaa Glu His Gly Asp Xaa
195 200 205
Xaa Pro Phe Asp Gly Pro Gly Gly Xaa Leu Ala His Ala Phe Xaa Pro
210 215 220
Gly Pro Gly Ile Gly Gly Asp Ala His Phe Asp Asp Asp Glu Xaa Trp
225 230 235 240
Thr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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260 265 270
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385 390 395 400
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
405 410 415
Xaa Xaa Xaa Xaa Xaa Gly Xaa Asn Leu Phe Leu Val Ala Ala His Glu
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Xaa Gly His Ser Leu Gly Leu Xaa His Ser Xaa Asp Pro Xaa Ala Leu
435 440 445
Met Tyr Pro Xaa Tyr Xaa Xaa Phe Xaa Asp Xaa Xaa Xaa Phe Xaa Leu
450 455 460
Xaa Xaa Asp Asp Ile Xaa Gly Ile Gln Xaa Leu Tyr Gly Xaa Xaa Xaa
465 470 475 480
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
485 490 495
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
500 505 510
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
515 520 525

```
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Pro Xaa Xaa Cys Asp Xaa Xaa Xaa
    530                 535                 540
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545                 550                 555                 560
Arg Gly Glu Xaa Phe Phe Phe Lys Asp Arg Phe Phe Trp Arg Xaa Xaa
                565                 570                 575
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Ile Xaa Xaa Phe
            580                 585                 590
Trp Pro Xaa Leu Pro Xaa Xaa Ile Asp Ala Ala Tyr Glu Xaa Xaa Xaa
        595                 600                 605
Xaa Xaa Xaa Val Phe Phe Phe Lys Gly Xaa Xaa Tyr Trp Xaa Tyr Xaa
    610                 615                 620
Gly Xaa Xaa Xaa Xaa Xaa Gly Tyr Pro Xaa Xaa Ile Xaa Xaa Xaa Leu
625                 630                 635                 640
Gly Phe Pro Xaa Xaa Val Xaa Xaa Ile Asp Ala Ala Xaa Xaa Xaa Xaa
                645                 650                 655
Xaa Xaa Xaa Xaa Lys Thr Tyr Phe Phe Xaa Xaa Xaa Xaa Tyr Trp Arg
            660                 665                 670
Tyr Asp Glu Xaa Xaa Xaa Xaa Met Asp Pro Gly Tyr Pro Lys Xaa Ile
        675                 680                 685
Xaa Xaa Xaa Phe Xaa Gly Ile Xaa Xaa Xaa Val Asp Ala Val Phe Xaa
    690                 695                 700
Xaa Xaa Xaa Xaa Gly Phe Xaa Tyr Phe Phe Xaa Gly Xaa Xaa Xaa Tyr
705                 710                 715                 720
Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                725                 730                 735
Xaa Xaa Xaa Xaa Trp Leu Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            740                 745                 750
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        755                 760                 765
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    770                 775                 780
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
785                 790                 795                 800
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                805                 810                 815
Xaa Xaa Xaa Xaa
            820
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Pro Arg Cys Gly Val Thr Asp
 1               5
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Arg Lys Lys Arg
1

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 469 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Ala Pro Thr Ser Thr Arg Phe Ser Asp Ala Ile Arg Ala Phe Gln
1 5 10 15
Trp Val Ser Gln Leu Pro Val Ser Gly Val Leu Asp Arg Ala Asn Leu
20 25 30
Arg Gln Met Thr Arg Pro Arg Cys Gly Val Thr Asp Thr Asn Ser Tyr
35 40 45
Ala Ala Trp Ala Glu Arg Ile Ser Asp Leu Phe Ala Arg His Arg Thr
50 55 60
Lys Met Arg Arg Lys Lys Arg Phe Ala Lys Gln Gly Asn Lys Trp Tyr
65 70 75 80
Lys Gln His Leu Ser Tyr Arg Leu Val Asn Trp Pro Glu His Leu Pro
85 90 95
Glu Pro Ala Val Arg Gly Ala Val Arg Ala Ala Phe Gln Leu Trp Ser
100 105 110
Asn Val Ser Ala Leu Glu Phe Trp Glu Ala Pro Ala Thr Gly Pro Ala
115 120 125
Asp Ile Arg Leu Thr Phe Phe Gln Gly Asp His Asn Asp Gly Leu Gly
130 135 140
Asn Ala Phe Asp Gly Pro Gly Gly Ala Leu Ala His Ala Phe Leu Pro
145 150 155 160
Arg Arg Gly Glu Ala His Phe Asp Gln Asp Glu Arg Trp Ser Leu Ser
165 170 175
Arg Arg Arg Gly Arg Asn Leu Phe Val Val Leu Ala His Glu Ile Gly
180 185 190
His Thr Leu Gly Leu Thr His Ser Pro Ala Pro Arg Ala Leu Met Ala
195 200 205

```
Pro Tyr Tyr Lys Arg Leu Gly Arg Asp Ala Leu Leu Ser Trp Asp Asp
    210                 215                 220
Val Leu Ala Val Gln Ser Leu Tyr Gly Lys Pro Leu Gly Gly Ser Val
225                 230                 235                 240
Ala Val Gln Leu Pro Gly Lys Leu Phe Thr Asp Phe Glu Thr Trp Asp
                245                 250                 255
Ser Tyr Ser Pro Gln Gly Arg Arg Pro Glu Thr Gln Gly Pro Lys Tyr
            260                 265                 270
Cys His Ser Ser Phe Asp Ala Ile Thr Val Asp Arg Gln Gln Gln Leu
        275                 280                 285
Tyr Ile Phe Lys Gly Ser His Phe Trp Glu Val Ala Ala Asp Gly Asn
    290                 295                 300
Val Ser Glu Pro Arg Pro Leu Gln Glu Arg Trp Val Gly Leu Pro Pro
305                 310                 315                 320
Asn Ile Glu Ala Ala Ala Val Ser Leu Asn Asp Gly Asp Phe Tyr Phe
                325                 330                 335
Phe Lys Gly Gly Arg Cys Trp Arg Phe Arg Gly Pro Lys Pro Val Trp
            340                 345                 350
Gly Leu Pro Gln Leu Cys Arg Ala Gly Gly Leu Pro Arg His Pro Asp
        355                 360                 365
Ala Ala Leu Phe Phe Pro Pro Leu Arg Arg Leu Ile Leu Phe Lys Gly
    370                 375                 380
Ala Arg Tyr Tyr Val Leu Ala Arg Gly Gly Leu Gln Val Glu Pro Tyr
385                 390                 395                 400
Tyr Pro Arg Ser Leu Gln Asp Trp Gly Gly Ile Pro Glu Glu Val Ser
                405                 410                 415
Gly Ala Leu Pro Arg Pro Asp Gly Ser Ile Ile Phe Phe Arg Asp Asp
            420                 425                 430
Arg Tyr Trp Arg Leu Asp Gln Ala Lys Leu Gln Ala Thr Thr Ser Gly
        435                 440                 445
Arg Trp Ala Thr Glu Leu Pro Trp Met Gly Cys Trp His Ala Asn Ser
    450                 455                 460
Gly Ser Ala Leu Phe
465
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Pro Arg Cys Gly Val Pro Asp
 1               5
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

His Glu Xaa Gly His Xaa Leu Gly Leu Xaa His Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gly Glu Gly Pro Leu Gly Leu Tyr Ala Lys Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Arg His Arg Thr Lys Met Arg Arg Lys Lys Arg Phe Ala Lys Gln Gly
1 5 10 15
Asn

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Phe Gln Gly Asp His Asn Asp Gly Leu Gly Asn Ala Phe Asp Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Arg Ser Leu Gln Asp Trp Gly Gly Ile Pro Glu Glu Val Ser Gly Ala
1 5 10 15
Leu Pro Arg Pro Asp Gly Ser Ile Ile
20 25

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Thr Glu Leu Pro Trp Met Gly Cys Trp His Ala Asn Ser Gly Ser
1 5 10 15
Ala Leu Phe

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 508 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Asn Cys Gln Gln Leu Trp Leu Gly Phe Leu Leu Pro Met Thr Val
1 5 10 15

Ser Gly Arg Val Leu Gly Leu Ala Glu Val Ala Pro Val Asp Tyr Leu
20 25 30
Ser Gln Tyr Gly Tyr Leu Gln Lys Pro Leu Glu Gly Ser Asn Asn Phe
35 40 45
Lys Pro Glu Asp Ile Thr Glu Ala Leu Arg Ala Phe Gln Glu Ala Ser
50 55 60
Glu Leu Pro Val Ser Gly Gln Leu Asp Asp Ala Thr Arg Ala Arg Met
65 70 75 80
Arg Gln Pro Arg Cys Gly Leu Glu Asp Pro Phe Asn Gln Lys Thr Leu
85 90 95
Lys Tyr Leu Leu Leu Gly Arg Trp Arg Lys Lys His Leu Thr Phe Arg
100 105 110
Ile Leu Asn Leu Pro Ser Thr Leu Pro Pro His Thr Ala Arg Ala Ala
115 120 125
Leu Arg Gln Ala Phe Gln Asp Trp Ser Asn Val Ala Pro Leu Thr Phe
130 135 140
Gln Glu Val Gln Ala Gly Ala Ala Asp Ile Arg Leu Ser Phe His Gly
145 150 155 160
Arg Gln Ser Ser Tyr Cys Ser Asn Thr Phe Asp Gly Pro Gly Arg Val
165 170 175
Leu Ala His Ala Asp Ile Pro Glu Leu Gly Ser Val His Phe Asp Glu
180 185 190
Asp Glu Phe Trp Thr Glu Gly Thr Tyr Arg Gly Val Asn Leu Arg Ile
195 200 205
Ile Ala Ala His Glu Val Gly His Ala Leu Gly Leu Gly His Ser Arg
210 215 220
Tyr Ser Gln Ala Leu Met Ala Pro Val Tyr Glu Gly Tyr Arg Pro His
225 230 235 240
Phe Lys Leu His Pro Asp Asp Val Ala Gly Ile Gln Ala Leu Tyr Gly
245 250 255
Lys Lys Ser Pro Val Ile Arg Asp Glu Glu Glu Glu Glu Thr Glu Leu
260 265 270
Pro Thr Val Pro Pro Val Pro Thr Glu Pro Ser Pro Met Pro Asp Pro
275 280 285
Cys Ser Ser Glu Leu Asp Ala Met Met Leu Gly Pro Arg Gly Lys Thr
290 295 300
Tyr Ala Phe Lys Gly Asp Tyr Val Trp Thr Val Ser Asp Ser Gly Pro
305 310 315 320
Gly Pro Leu Phe Arg Val Ser Ala Leu Trp Glu Gly Leu Pro Gly Asn
325 330 335
Leu Asp Ala Ala Val Tyr Ser Pro Arg Thr Gln Trp Ile His Phe Phe
340 345 350
Lys Gly Asp Lys Val Trp Arg Tyr Ile Asn Phe Lys Met Ser Pro Gly
355 360 365
Phe Pro Lys Lys Leu Asn Arg Ser Glu Pro Asn Leu Asp Ala Ala Leu
370 375 380
Tyr Trp Pro Leu Asn Gln Lys Val Phe Leu Phe Lys Gly Ser Gly Tyr
385 390 395 400
Trp Gln Trp Asp Glu Leu Ala Arg Thr Asp Phe Ser Ser Tyr Pro Lys
405 410 415
Pro Ile Lys Gly Leu Phe Thr Gly Val Pro Asn Gln Pro Ser Ala Ala
420 425 430
Met Ser Trp Gln Asp Gly Arg Val Tyr Phe Phe Lys Gly Lys Val Tyr

435 440 445
Trp Arg Leu Asn Gln Gln Leu Arg Val Glu Lys Gly Tyr Pro Arg Asn
450 455 460
Ile Ser His Asn Trp Met His Cys Arg Pro Arg Thr Ile Asp Thr Thr
465 470 475 480
Pro Ser Gly Gly Asn Thr Thr Pro Ser Gly Thr Gly Ile Thr Leu Asp
485 490 495
Thr Thr Leu Ser Ala Thr Glu Thr Thr Phe Glu Tyr
500 505

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 660 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Glu Ala Leu Met Ala Arg Gly Ala Leu Thr Gly Pro Leu Arg Ala
1 5 10 15
Leu Cys Leu Leu Gly Cys Leu Leu Ser His Ala Ala Ala Ala Pro Ser
20 25 30
Pro Ile Ile Lys Phe Pro Gly Asp Val Ala Pro Lys Thr Asp Lys Glu
35 40 45
Leu Ala Val Gln Tyr Leu Asn Thr Phe Tyr Gly Cys Pro Lys Glu Ser
50 55 60
Cys Asn Leu Phe Val Leu Lys Asp Thr Leu Lys Lys Met Gln Lys Phe
65 70 75 80
Phe Gly Leu Pro Gln Thr Gly Asp Leu Asp Gln Asn Thr Ile Glu Thr
85 90 95
Met Arg Lys Pro Arg Cys Gly Asn Pro Asp Val Ala Asn Tyr Asn Phe
100 105 110
Phe Pro Arg Lys Pro Lys Trp Asp Lys Asn Gln Ile Thr Tyr Arg Ile
115 120 125
Ile Gly Tyr Thr Pro Asp Leu Asp Pro Glu Thr Val Asp Asp Ala Phe
130 135 140
Ala Arg Ala Phe Gln Val Trp Ser Asp Val Thr Pro Leu Arg Phe Ser
145 150 155 160
Arg Ile His Asp Gly Glu Ala Asp Ile Met Ile Asn Phe Gly Arg Trp
165 170 175
Glu His Gly Asp Gly Tyr Pro Phe Asp Gly Lys Asp Gly Leu Leu Ala
180 185 190
His Ala Phe Ala Pro Gly Thr Gly Val Gly Gly Asp Ser His Phe Asp
195 200 205
Asp Asp Glu Leu Trp Thr Leu Gly Glu Gly Gln Val Val Arg Val Lys
210 215 220
Tyr Gly Asn Ala Asp Gly Glu Tyr Cys Lys Phe Pro Phe Leu Phe Asn
225 230 235 240
Gly Lys Glu Tyr Asn Ser Cys Thr Asp Thr Gly Arg Ser Asp Gly Phe

245 250 255
Leu Trp Cys Ser Thr Thr Tyr Asn Phe Glu Lys Asp Gly Lys Tyr Gly
260 265 270
Phe Cys Pro His Glu Ala Leu Phe Thr Met Gly Gly Asn Ala Glu Gly
275 280 285
Gln Pro Cys Lys Phe Pro Phe Arg Phe Gln Gly Thr Ser Tyr Asp Ser
290 295 300
Cys Thr Thr Glu Gly Arg Thr Asp Gly Tyr Arg Trp Cys Gly Thr Thr
305 310 315 320
Glu Asp Tyr Asp Arg Asp Lys Lys Tyr Gly Phe Cys Pro Glu Thr Ala
325 330 335
Met Ser Thr Val Gly Gly Asn Ser Glu Gly Ala Pro Cys Val Phe Pro
340 345 350
Phe Thr Phe Leu Gly Asn Lys Tyr Glu Ser Cys Thr Ser Ala Gly Arg
355 360 365
Ser Asp Gly Lys Met Trp Cys Ala Thr Thr Ala Asn Tyr Asp Asp Asp
370 375 380
Arg Lys Trp Gly Phe Cys Pro Asp Gln Gly Tyr Ser Leu Phe Leu Val
385 390 395 400
Ala Ala His Glu Phe Gly His Ala Met Gly Leu Glu His Ser Gln Asp
405 410 415
Pro Gly Ala Leu Met Ala Pro Ile Tyr Thr Tyr Thr Lys Asn Phe Arg
420 425 430
Leu Ser Gln Asp Asp Ile Lys Gly Ile Gln Glu Leu Tyr Gly Ala Ser
435 440 445
Pro Asp Ile Asp Leu Gly Thr Gly Pro Thr Pro Thr Leu Gly Pro Val
450 455 460
Thr Pro Glu Ile Cys Lys Gln Asp Ile Val Phe Asp Gly Ile Ala Gln
465 470 475 480
Ile Arg Gly Glu Ile Phe Phe Phe Lys Asp Arg Phe Ile Trp Arg Thr
485 490 495
Val Thr Pro Arg Asp Lys Pro Met Gly Pro Leu Leu Val Ala Thr Phe
500 505 510
Trp Pro Glu Leu Pro Glu Lys Ile Asp Ala Val Tyr Glu Ala Pro Gln
515 520 525
Glu Glu Lys Ala Val Phe Phe Ala Gly Asn Glu Tyr Trp Ile Tyr Ser
530 535 540
Ala Ser Thr Leu Glu Arg Gly Tyr Pro Lys Pro Leu Thr Ser Leu Gly
545 550 555 560
Leu Pro Pro Asp Val Gln Arg Val Asp Ala Ala Phe Asn Trp Ser Lys
565 570 575
Asn Lys Lys Thr Tyr Ile Phe Ala Gly Asp Lys Phe Trp Arg Tyr Asn
580 585 590
Glu Val Lys Lys Lys Met Asp Pro Gly Phe Pro Lys Leu Ile Ala Asp
595 600 605
Ala Trp Asn Ala Ile Pro Asp Asn Leu Asp Ala Val Val Asp Leu Gln
610 615 620
Gly Gly Gly His Ser Tyr Phe Phe Lys Gly Ala Tyr Tyr Leu Lys Leu
625 630 635 640
Glu Asn Gln Ser Leu Lys Ser Val Lys Phe Gly Ser Ile Lys Ser Asp
645 650 655
Trp Leu Gly Cys
660

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 707 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Ser Leu Trp Gln Pro Leu Val Leu Val Leu Leu Val Leu Gly Cys
1 5 10 15
Cys Phe Ala Ala Pro Arg Gln Arg Gln Ser Thr Leu Val Leu Phe Pro
20 25 30
Gly Asp Leu Arg Thr Asn Leu Thr Asp Arg Gln Leu Ala Glu Glu Tyr
35 40 45
Leu Tyr Arg Tyr Gly Tyr Thr Arg Val Ala Glu Met Arg Gly Glu Ser
50 55 60
Lys Ser Leu Gly Pro Ala Leu Leu Leu Leu Gln Lys Gln Leu Ser Leu
65 70 75 80
Pro Glu Thr Gly Glu Leu Asp Ser Ala Thr Leu Lys Ala Met Arg Thr
85 90 95
Pro Arg Cys Gly Val Pro Asp Leu Gly Arg Phe Gln Thr Phe Glu Gly
100 105 110
Asp Leu Lys Trp His His His Asn Ile Thr Tyr Trp Ile Gln Asn Tyr
115 120 125
Ser Glu Asp Leu Pro Arg Ala Val Ile Asp Asp Ala Phe Ala Arg Ala
130 135 140
Phe Ala Leu Trp Ser Ala Val Thr Pro Leu Thr Phe Thr Arg Val Tyr
145 150 155 160
Ser Arg Asp Ala Asp Ile Val Ile Gln Phe Gly Val Ala Glu His Gly
165 170 175
Asp Gly Tyr Pro Phe Asp Gly Lys Asp Gly Leu Leu Ala His Ala Phe
180 185 190
Pro Pro Gly Pro Gly Ile Gln Gly Asp Ala His Phe Asp Asp Asp Glu
195 200 205
Leu Trp Ser Leu Gly Lys Gly Val Val Val Pro Thr Arg Phe Gly Asn
210 215 220
Ala Asp Gly Ala Ala Cys His Phe Pro Phe Ile Phe Glu Gly Arg Ser
225 230 235 240
Tyr Ser Ala Cys Thr Thr Asp Gly Arg Ser Asp Gly Leu Pro Trp Cys
245 250 255
Ser Thr Thr Ala Asn Tyr Asp Thr Asp Asp Arg Phe Gly Phe Cys Pro
260 265 270
Ser Glu Arg Leu Tyr Thr Arg Asp Gly Asn Ala Asp Gly Lys Pro Cys
275 280 285
Gln Phe Pro Phe Ile Phe Gln Gly Gln Ser Tyr Ser Ala Cys Thr Thr
290 295 300
Asp Gly Arg Ser Asp Gly Tyr Arg Trp Cys Ala Thr Thr Ala Asn Tyr
305 310 315 320
Asp Arg Asp Lys Leu Phe Gly Phe Cys Pro Thr Arg Ala Asp Ser Thr

325 330 335
Val Met Gly Gly Asn Ser Ala Gly Glu Leu Cys Val Phe Pro Phe Thr
340 345 350
Phe Leu Gly Lys Glu Tyr Ser Thr Cys Thr Ser Glu Gly Arg Gly Asp
355 360 365
Gly Arg Leu Trp Cys Ala Thr Thr Ser Asn Phe Asp Ser Asp Lys Lys
370 375 380
Trp Gly Phe Cys Pro Asp Gln Gly Tyr Ser Leu Phe Leu Val Ala Ala
385 390 395 400
His Glu Phe Gly His Ala Leu Gly Leu Asp His Ser Ser Val Pro Glu
405 410 415
Ala Leu Met Tyr Pro Met Tyr Arg Phe Thr Glu Gly Pro Pro Leu His
420 425 430
Lys Asp Asp Val Asn Gly Ile Arg His Leu Tyr Gly Pro Arg Pro Glu
435 440 445
Pro Glu Pro Arg Pro Pro Thr Thr Thr Thr Pro Gln Pro Thr Ala Pro
450 455 460
Pro Thr Val Cys Pro Thr Gly Pro Pro Thr Val His Pro Ser Glu Arg
465 470 475 480
Pro Thr Ala Gly Pro Thr Gly Pro Pro Ser Ala Gly Pro Thr Gly Pro
485 490 495
Pro Thr Ala Gly Pro Ser Thr Ala Thr Thr Val Pro Leu Ser Pro Val
500 505 510
Asp Asp Ala Cys Asn Val Asn Ile Phe Asp Ala Ile Ala Glu Ile Gly
515 520 525
Asn Gln Leu Tyr Leu Phe Lys Asp Gly Lys Tyr Trp Arg Phe Ser Glu
530 535 540
Gly Arg Gly Ser Arg Pro Gln Gly Pro Phe Leu Ile Ala Asp Lys Trp
545 550 555 560
Pro Ala Leu Pro Arg Lys Leu Asp Ser Val Phe Glu Glu Pro Leu Ser
565 570 575
Lys Lys Leu Phe Phe Phe Ser Gly Arg Gln Val Trp Val Tyr Thr Gly
580 585 590
Ala Ser Val Leu Gly Pro Arg Arg Leu Asp Lys Leu Gly Leu Gly Ala
595 600 605
Asp Val Ala Gln Val Thr Gly Ala Leu Arg Ser Gly Arg Gly Lys Met
610 615 620
Leu Leu Phe Ser Gly Arg Arg Leu Trp Arg Phe Asp Val Lys Ala Gln
625 630 635 640
Met Val Asp Pro Arg Ser Ala Ser Glu Val Asp Arg Met Phe Pro Gly
645 650 655
Val Pro Leu Asp Thr His Asp Val Phe Gln Tyr Arg Glu Lys Ala Tyr
660 665 670
Phe Cys Gln Asp Arg Phe Tyr Trp Arg Val Ser Ser Arg Ser Glu Leu
675 680 685
Asn Gln Val Asp Gln Val Gly Tyr Val Thr Tyr Asp Ile Leu Gln Cys
690 695 700
Pro Glu Asp
705

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 477 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Met Lys Ser Leu Pro Ile Leu Leu Leu Leu Cys Val Ala Val Cys Ser
 1               5                  10                  15
Ala Tyr Pro Leu Asp Gly Ala Ala Arg Gly Glu Asp Thr Ser Met Asn
            20                  25                  30
Leu Val Gln Lys Tyr Leu Glu Asn Tyr Tyr Asp Leu Lys Lys Asp Val
        35                  40                  45
Lys Gln Phe Val Arg Arg Lys Asp Ser Gly Pro Val Val Lys Lys Ile
    50                  55                  60
Arg Glu Met Gln Lys Phe Leu Gly Leu Glu Val Thr Gly Lys Leu Asp
65                  70                  75                  80
Ser Asp Thr Leu Glu Val Met Arg Lys Pro Arg Cys Gly Val Pro Asp
                85                  90                  95
Val Gly His Phe Arg Thr Phe Pro Gly Ile Pro Lys Trp Arg Lys Thr
            100                 105                 110
His Leu Thr Tyr Arg Ile Val Asn Tyr Thr Pro Asp Leu Pro Lys Asp
        115                 120                 125
Ala Val Asp Ser Ala Val Glu Lys Ala Leu Lys Val Trp Glu Glu Val
    130                 135                 140
Thr Pro Leu Thr Phe Ser Arg Leu Tyr Glu Gly Glu Ala Asp Ile Met
145                 150                 155                 160
Ile Ser Phe Ala Val Arg Glu His Gly Asp Phe Tyr Pro Phe Asp Gly
                165                 170                 175
Pro Gly Asn Val Leu Ala His Ala Tyr Ala Pro Gly Pro Gly Ile Asn
            180                 185                 190
Gly Asp Ala His Phe Asp Asp Asp Glu Gln Trp Thr Lys Asp Thr Thr
        195                 200                 205
Gly Thr Asn Leu Phe Leu Val Ala Ala His Glu Ile Gly His Ser Leu
    210                 215                 220
Gly Leu Phe His Ser Ala Asn Thr Glu Ala Leu Met Tyr Pro Leu Tyr
225                 230                 235                 240
His Ser Leu Thr Asp Leu Thr Arg Phe Arg Leu Ser Gln Asp Asp Ile
                245                 250                 255
Asn Gly Ile Gln Ser Leu Tyr Gly Pro Pro Pro Asp Ser Pro Glu Thr
            260                 265                 270
Pro Leu Val Pro Thr Glu Pro Val Pro Pro Glu Pro Gly Thr Pro Ala
        275                 280                 285
Asn Cys Asp Pro Ala Leu Ser Phe Asp Ala Val Ser Thr Leu Arg Gly
    290                 295                 300
Glu Ile Leu Ile Phe Lys Asp Arg His Phe Trp Arg Lys Ser Leu Arg
305                 310                 315                 320
Lys Leu Glu Pro Glu Leu His Leu Ile Ser Ser Phe Trp Pro Ser Leu
                325                 330                 335
Pro Ser Gly Val Asp Ala Ala Tyr Glu Val Thr Ser Lys Asp Leu Val
            340                 345                 350
```

```
Phe Ile Phe Lys Gly Asn Gln Phe Trp Ala Ile Arg Gly Asn Glu Val
        355                 360                 365
Arg Ala Gly Tyr Pro Arg Gly Ile His Thr Leu Gly Phe Pro Pro Thr
    370                 375                 380
Val Arg Lys Ile Asp Ala Ala Ile Ser Asp Lys Glu Lys Asn Lys Thr
385                 390                 395                 400
Tyr Phe Phe Val Glu Asp Lys Tyr Trp Arg Phe Asp Glu Lys Arg Asn
                405                 410                 415
Ser Met Glu Pro Gly Phe Pro Lys Gln Ile Ala Glu Asp Phe Pro Gly
            420                 425                 430
Ile Asp Ser Lys Ile Asp Ala Val Phe Glu Glu Phe Gly Phe Phe Tyr
        435                 440                 445
Phe Phe Thr Gly Ser Ser Gln Leu Glu Phe Asp Pro Asn Ala Lys Lys
    450                 455                 460
Val Thr His Thr Leu Lys Ser Asn Ser Trp Leu Asn Cys
465                 470                 475
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 476 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Met Met His Leu Ala Phe Leu Val Leu Leu Cys Leu Pro Val Cys Ser
 1               5                  10                  15
Ala Tyr Pro Leu Ser Gly Ala Ala Lys Glu Glu Asp Ser Asn Lys Asp
            20                  25                  30
Leu Ala Gln Gln Tyr Leu Glu Lys Tyr Tyr Asn Leu Glu Lys Asp Val
        35                  40                  45
Lys Gln Phe Arg Arg Lys Asp Ser Asn Leu Ile Val Lys Lys Ile Gln
    50                  55                  60
Gly Met Gln Lys Phe Leu Gly Leu Glu Val Thr Gly Lys Leu Asp Thr
65                  70                  75                  80
Asp Thr Leu Glu Val Met Arg Lys Pro Arg Cys Gly Val Pro Asp Val
                85                  90                  95
Gly His Phe Ser Ser Phe Pro Gly Met Pro Lys Trp Arg Lys Thr His
            100                 105                 110
Leu Thr Tyr Arg Ile Val Asn Tyr Thr Pro Asp Leu Pro Arg Asp Ala
        115                 120                 125
Val Asp Ser Ala Ile Glu Lys Ala Leu Lys Val Trp Glu Glu Val Thr
    130                 135                 140
Pro Leu Thr Phe Ser Arg Leu Tyr Glu Gly Glu Ala Asp Ile Met Ile
145                 150                 155                 160
Ser Phe Ala Val Lys Glu His Gly Asp Phe Tyr Ser Phe Asp Gly Pro
                165                 170                 175
Gly His Ser Leu Ala His Ala Tyr Pro Pro Gly Pro Gly Leu Tyr Gly
            180                 185                 190
```

```
Asp Ile His Phe Asp Asp Asp Glu Lys Trp Thr Glu Asp Ala Ser Gly
        195                 200                 205
Thr Asn Leu Phe Leu Val Ala Ala His Glu Leu Gly His Ser Leu Gly
    210                 215                 220
Leu Phe His Ser Ala Asn Thr Glu Ala Leu Met Tyr Pro Leu Tyr Asn
225                 230                 235                 240
Ser Phe Thr Glu Leu Ala Gln Phe Arg Leu Ser Gln Asp Asp Val Asn
                245                 250                 255
Gly Ile Gln Ser Leu Tyr Gly Pro Pro Pro Ala Ser Thr Glu Glu Pro
            260                 265                 270
Leu Val Pro Thr Lys Ser Val Pro Ser Gly Ser Glu Met Pro Ala Lys
        275                 280                 285
Cys Asp Pro Ala Leu Ser Phe Asp Ala Ile Ser Thr Leu Arg Gly Glu
    290                 295                 300
Tyr Leu Phe Phe Lys Asp Arg Tyr Phe Trp Arg Arg Ser His Trp Asn
305                 310                 315                 320
Pro Glu Pro Glu Phe His Leu Ile Ser Ala Phe Trp Pro Ser Leu Pro
                325                 330                 335
Ser Tyr Leu Asp Ala Ala Tyr Glu Val Asn Ser Arg Asp Thr Val Phe
            340                 345                 350
Ile Phe Lys Gly Asn Glu Phe Trp Ala Ile Arg Gly Asn Glu Val Gln
        355                 360                 365
Ala Gly Tyr Pro Arg Gly Ile His Thr Leu Gly Phe Pro Pro Thr Ile
    370                 375                 380
Arg Lys Ile Asp Ala Ala Val Ser Asp Lys Glu Lys Lys Lys Thr Tyr
385                 390                 395                 400
Phe Phe Ala Ala Asp Lys Tyr Trp Arg Phe Asp Glu Asn Ser Gln Ser
                405                 410                 415
Met Glu Gln Gly Phe Pro Arg Leu Ile Ala Asp Asp Phe Pro Gly Val
            420                 425                 430
Glu Pro Lys Val Asp Ala Val Leu Gln Ala Phe Gly Phe Phe Tyr Phe
        435                 440                 445
Phe Ser Gly Ser Ser Gln Phe Glu Phe Asp Pro Asn Ala Arg Met Val
    450                 455                 460
Thr His Ile Leu Lys Ser Asn Ser Trp Leu His Cys
465                 470                 475
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 469 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Met His Ser Phe Pro Pro Leu Leu Leu Leu Phe Trp Gly Val Val
 1               5                  10                  15
Ser His Ser Phe Pro Ala Thr Leu Glu Thr Gln Glu Gln Asp Val Asp
            20                  25                  30
```

```
Leu Val Gln Lys Tyr Leu Glu Lys Tyr Tyr Asn Leu Lys Asn Asp Gly
        35                  40                  45
Arg Gln Val Glu Lys Arg Arg Asn Ser Gly Pro Val Val Glu Lys Leu
    50                  55                  60
Lys Gln Met Gln Glu Phe Phe Gly Leu Lys Val Thr Gly Lys Pro Asp
65                  70                  75                  80
Ala Glu Thr Leu Lys Val Met Lys Gln Pro Arg Cys Gly Val Pro Asp
                85                  90                  95
Val Ala Gln Phe Val Leu Thr Glu Gly Asn Pro Arg Trp Glu Gln Thr
            100                 105                 110
His Leu Thr Tyr Arg Ile Glu Asn Tyr Thr Pro Asp Leu Pro Arg Ala
        115                 120                 125
Asp Val Asp His Ala Ile Glu Lys Ala Phe Gln Leu Trp Ser Asn Val
    130                 135                 140
Thr Pro Leu Thr Phe Thr Lys Val Ser Glu Gly Gln Ala Asp Ile Met
145                 150                 155                 160
Ile Ser Phe Val Arg Gly Asp His Arg Asp Asn Ser Pro Phe Asp Gly
                165                 170                 175
Pro Gly Gly Asn Leu Ala His Ala Phe Gln Pro Gly Pro Gly Ile Gly
            180                 185                 190
Gly Asp Ala His Phe Asp Glu Asp Glu Arg Trp Thr Asn Asn Phe Arg
        195                 200                 205
Glu Tyr Asn Leu His Arg Val Ala Ala His Glu Leu Gly His Ser Leu
    210                 215                 220
Gly Leu Ser His Ser Thr Asp Ile Gly Ala Leu Met Tyr Pro Ser Tyr
225                 230                 235                 240
Thr Phe Ser Gly Asp Val Gln Leu Ala Gln Asp Asp Ile Asp Gly Ile
                245                 250                 255
Gln Ala Ile Tyr Gly Arg Ser Gln Asn Pro Val Gln Pro Ile Gly Pro
            260                 265                 270
Gln Thr Pro Lys Ala Cys Asp Ser Lys Leu Thr Phe Asp Ala Ile Thr
        275                 280                 285
Thr Ile Arg Gly Glu Val Met Phe Phe Lys Asp Arg Phe Tyr Met Arg
    290                 295                 300
Thr Asn Pro Phe Tyr Pro Glu Val Glu Leu Asn Phe Ile Ser Val Phe
305                 310                 315                 320
Trp Pro Gln Leu Pro Asn Gly Leu Glu Ala Ala Tyr Glu Phe Ala Asp
                325                 330                 335
Arg Asp Glu Val Arg Phe Phe Lys Gly Asn Lys Tyr Trp Ala Val Gln
            340                 345                 350
Gly Gln Asn Val Leu His Gly Tyr Pro Lys Asp Ile Tyr Ser Ser Phe
        355                 360                 365
Gly Phe Pro Arg Thr Val Lys His Ile Asp Ala Ala Leu Ser Glu Glu
    370                 375                 380
Asn Thr Gly Lys Thr Tyr Phe Phe Val Ala Asn Lys Tyr Trp Arg Tyr
385                 390                 395                 400
Asp Glu Tyr Lys Arg Ser Met Asp Pro Gly Tyr Pro Lys Met Ile Ala
                405                 410                 415
His Asp Phe Pro Gly Ile Gly His Lys Val Asp Ala Val Phe Met Lys
            420                 425                 430
Asp Gly Phe Phe Tyr Phe Phe His Gly Thr Arg Gln Tyr Lys Phe Asp
        435                 440                 445
Pro Lys Thr Lys Arg Ile Leu Thr Leu Gln Lys Ala Asn Ser Trp Phe
```

450 455 460
Asn Cys Arg Lys Asn
465

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 467 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Phe Ser Leu Lys Thr Leu Pro Phe Leu Leu Leu Leu His Val Gln
1 5 10 15
Ile Ser Lys Ala Phe Pro Val Ser Ser Lys Glu Lys Asn Thr Lys Thr
20 25 30
Val Gln Asp Tyr Leu Glu Lys Phe Tyr Gln Leu Pro Ser Asn Gln Tyr
35 40 45
Gln Ser Thr Arg Lys Asn Gly Thr Asn Val Ile Val Glu Lys Leu Lys
50 55 60
Glu Met Gln Arg Phe Phe Gly Leu Asn Val Thr Gly Lys Pro Asn Glu
65 70 75 80
Glu Thr Leu Asp Met Met Lys Lys Pro Arg Cys Gly Val Pro Asp Ser
85 90 95
Gly Gly Phe Met Leu Thr Pro Gly Asn Pro Lys Trp Glu Arg Thr Asn
100 105 110
Leu Thr Tyr Arg Ile Arg Asn Tyr Thr Pro Gln Leu Ser Glu Ala Glu
115 120 125
Val Glu Arg Ala Ile Lys Asp Ala Phe Glu Leu Trp Ser Val Ala Ser
130 135 140
Pro Leu Ile Phe Thr Arg Ile Ser Gln Gly Glu Ala Asp Ile Asn Ile
145 150 155 160
Ala Phe Tyr Gln Arg Asp His Gly Asp Asn Ser Pro Phe Asp Gly Pro
165 170 175
Asn Gly Ile Leu Ala His Ala Phe Gln Pro Gly Gln Gly Ile Gly Gly
180 185 190
Asp Ala His Phe Asp Ala Glu Glu Thr Trp Thr Asn Thr Ser Ala Asn
195 200 205
Tyr Asn Leu Phe Leu Val Ala Ala His Glu Phe Gly His Ser Leu Gly
210 215 220
Leu Ala His Ser Ser Asp Pro Gly Ala Leu Met Tyr Pro Asn Tyr Ala
225 230 235 240
Phe Arg Glu Thr Ser Asn Tyr Ser Leu Pro Gln Asp Asp Ile Asp Gly
245 250 255
Ile Gln Ala Ile Tyr Gly Leu Ser Ser Asn Pro Ile Gln Pro Thr Gly
260 265 270
Pro Ser Thr Pro Lys Pro Cys Asp Pro Ser Leu Thr Phe Asp Ala Ile
275 280 285
Thr Thr Leu Arg Gly Glu Ile Leu Phe Phe Lys Asp Arg Tyr Phe Trp

```
    290                 295                 300
Arg Arg His Pro Gln Leu Gln Arg Val Glu Met Asn Phe Ile Ser Leu
305                 310                 315                 320
Phe Trp Pro Ser Leu Pro Thr Gly Ile Gln Ala Ala Tyr Glu Asp Phe
                325                 330                 335
Asp Arg Asp Leu Ile Phe Leu Phe Lys Gly Asn Gln Tyr Trp Ala Leu
            340                 345                 350
Ser Gly Tyr Asp Ile Leu Gln Gly Tyr Pro Lys Asp Ile Ser Asn Tyr
        355                 360                 365
Gly Phe Pro Ser Ser Val Gln Ala Ile Asp Ala Ala Val Phe Tyr Arg
    370                 375                 380
Ser Lys Thr Tyr Phe Phe Val Asn Asp Gln Phe Trp Arg Tyr Asp Asn
385                 390                 395                 400
Gln Arg Gln Phe Met Glu Pro Gly Tyr Pro Lys Ser Ile Ser Gly Ala
                405                 410                 415
Phe Pro Gly Ile Glu Ser Lys Val Asp Ala Val Phe Gln Gln Glu His
            420                 425                 430
Phe Phe His Val Phe Ser Gly Pro Arg Tyr Tyr Ala Phe Asp Leu Ile
        435                 440                 445
Ala Gln Arg Val Thr Arg Val Ala Arg Gly Asn Lys Trp Leu Asn Cys
    450                 455                 460
Arg Tyr Gly
465
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 471 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Met His Pro Gly Val Leu Ala Ala Phe Leu Phe Leu Ser Trp Thr His
 1               5                  10                  15
Cys Arg Ala Leu Pro Leu Pro Ser Gly Gly Asp Glu Asp Asp Leu Ser
            20                  25                  30
Glu Glu Asp Leu Gln Phe Ala Glu Arg Tyr Leu Arg Ser Tyr Tyr His
        35                  40                  45
Pro Thr Asn Leu Ala Gly Ile Leu Lys Glu Asn Ala Ala Ser Ser Met
    50                  55                  60
Thr Glu Arg Leu Arg Glu Met Gln Ser Phe Phe Gly Leu Glu Val Thr
65                  70                  75                  80
Gly Lys Leu Asp Asp Asn Thr Leu Asp Val Met Lys Lys Pro Arg Cys
                85                  90                  95
Gly Val Pro Asp Val Gly Glu Tyr Asn Val Phe Pro Arg Thr Leu Lys
            100                 105                 110
Trp Ser Lys Met Asn Leu Thr Tyr Arg Ile Val Asn Tyr Thr Pro Asp
        115                 120                 125
Met Thr His Ser Glu Val Glu Lys Ala Phe Lys Lys Ala Phe Lys Val
```

```
    130                 135                 140
Trp Ser Asp Val Thr Pro Leu Asn Phe Thr Arg Leu His Asp Gly Ile
145                 150                 155                 160
Ala Asp Ile Met Ile Ser Phe Gly Ile Lys Glu His Gly Asp Phe Tyr
                165                 170                 175
Pro Phe Asp Gly Pro Ser Gly Leu Leu Ala His Ala Phe Pro Pro Gly
            180                 185                 190
Pro Asn Tyr Gly Gly Asp Ala His Phe Asp Asp Asp Glu Thr Trp Thr
        195                 200                 205
Ser Ser Ser Lys Gly Tyr Asn Leu Phe Leu Val Ala Ala His Glu Phe
    210                 215                 220
Gly His Ser Leu Gly Leu Asp His Ser Lys Asp Pro Gly Ala Leu Met
225                 230                 235                 240
Phe Pro Ile Tyr Thr Tyr Thr Gly Lys Ser His Phe Met Leu Pro Asp
                245                 250                 255
Asp Asp Val Gln Gly Ile Gln Ser Leu Tyr Gly Pro Gly Asp Glu Asp
            260                 265                 270
Pro Asn Pro Lys His Pro Lys Thr Pro Asp Lys Cys Asp Pro Ser Leu
        275                 280                 285
Ser Leu Asp Ala Ile Thr Ser Leu Arg Gly Glu Thr Met Ile Phe Lys
    290                 295                 300
Asp Arg Phe Phe Trp Arg Leu His Pro Gln Gln Val Asp Ala Glu Leu
305                 310                 315                 320
Phe Leu Thr Lys Ser Phe Trp Pro Glu Leu Pro Asn Arg Ile Asp Ala
                325                 330                 335
Ala Tyr Glu His Pro Ser His Asp Leu Ile Phe Ile Phe Arg Gly Arg
            340                 345                 350
Lys Phe Trp Ala Leu Asn Gly Tyr Asp Ile Leu Glu Gly Tyr Pro Lys
        355                 360                 365
Lys Ile Ser Glu Leu Gly Leu Pro Lys Glu Val Lys Lys Ile Ser Ala
    370                 375                 380
Ala Val His Phe Glu Asp Thr Gly Lys Thr Leu Leu Phe Ser Gly Asn
385                 390                 395                 400
Gln Val Trp Arg Tyr Asp Asp Thr Asn His Ile Met Asp Lys Asp Tyr
                405                 410                 415
Pro Arg Leu Ile Glu Glu Asp Phe Pro Gly Ile Gly Asp Lys Val Asp
            420                 425                 430
Ala Val Tyr Glu Lys Asn Gly Tyr Ile Tyr Phe Phe Asn Gly Pro Ile
        435                 440                 445
Gln Phe Glu Tyr Ser Ile Trp Ser Asn Arg Ile Val Arg Val Met Pro
    450                 455                 460
Ala Asn Ser Ile Leu Trp Cys
465                 470
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 470 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Met Lys Phe Leu Leu Ile Leu Leu Gln Ala Thr Ala Ser Gly Ala
 1               5                  10                  15
Leu Pro Leu Asn Ser Ser Thr Ser Leu Glu Lys Asn Asn Val Leu Phe
            20                  25                  30
Gly Glu Arg Tyr Leu Glu Lys Phe Tyr Gly Leu Glu Ile Asn Lys Leu
        35                  40                  45
Pro Val Thr Lys Met Lys Tyr Ser Gly Asn Leu Met Lys Glu Lys Ile
    50                  55                  60
Gln Glu Met Gln His Phe Leu Gly Leu Lys Val Thr Gly Gln Leu Asp
65                  70                  75                  80
Thr Ser Thr Leu Glu Met Met His Ala Pro Arg Cys Gly Val Pro Asp
                85                  90                  95
Val His His Phe Arg Glu Met Pro Gly Gly Pro Val Trp Arg Lys His
            100                 105                 110
Tyr Ile Thr Tyr Arg Ile Asn Asn Tyr Thr Pro Asp Met Asn Arg Glu
        115                 120                 125
Asp Val Asp Tyr Ala Ile Arg Lys Ala Phe Gln Val Trp Ser Asn Val
    130                 135                 140
Thr Pro Leu Lys Phe Ser Lys Ile Asn Thr Gly Met Ala Asp Ile Leu
145                 150                 155                 160
Val Val Phe Ala Arg Gly Ala His Gly Asp Phe His Ala Phe Asp Gly
                165                 170                 175
Lys Gly Gly Ile Leu Ala His Ala Phe Gly Pro Gly Ser Gly Ile Gly
            180                 185                 190
Gly Asp Ala His Phe Asp Glu Asp Glu Phe Trp Thr Thr His Ser Gly
        195                 200                 205
Gly Thr Asn Leu Phe Leu Thr Ala Val His Glu Ile Gly His Ser Leu
    210                 215                 220
Gly Leu Gly His Ser Ser Asp Pro Lys Ala Val Met Phe Pro Thr Tyr
225                 230                 235                 240
Lys Tyr Val Asp Ile Asn Thr Phe Arg Leu Ser Ala Asp Asp Ile Arg
                245                 250                 255
Gly Ile Gln Ser Leu Tyr Gly Asp Pro Lys Glu Asn Gln Arg Leu Pro
            260                 265                 270
Asn Pro Asp Asn Ser Glu Pro Ala Leu Cys Asp Pro Asn Leu Ser Phe
        275                 280                 285
Asp Ala Val Thr Thr Val Gly Asn Lys Ile Phe Phe Phe Lys Asp Arg
    290                 295                 300
Phe Phe Trp Leu Lys Val Ser Glu Arg Pro Lys Thr Ser Val Asn Leu
305                 310                 315                 320
Ile Ser Ser Leu Trp Pro Thr Leu Pro Ser Gly Ile Glu Ala Ala Tyr
                325                 330                 335
Glu Ile Glu Ala Arg Asn Gln Val Phe Leu Phe Lys Asp Asp Lys Tyr
            340                 345                 350
Trp Leu Ile Ser Asn Leu Arg Pro Glu Pro Asn Tyr Pro Lys Ser Ile
        355                 360                 365
His Ser Phe Gly Phe Pro Asn Phe Val Lys Lys Ile Asp Ala Ala Val
    370                 375                 380
Phe Asn Pro Arg Phe Tyr Arg Thr Tyr Phe Phe Val Asp Asn Gln Tyr
385                 390                 395                 400
Trp Arg Tyr Asp Glu Arg Arg Gln Met Met Asp Pro Gly Tyr Pro Lys
```

```
                405                 410                 415
Leu Ile Thr Lys Asn Phe Gln Gly Ile Gly Pro Lys Ile Asp Ala Val
            420                 425                 430
Phe Tyr Ser Lys Asn Lys Tyr Tyr Tyr Phe Phe Gln Gly Ser Asn Gln
        435                 440                 445
Phe Glu Tyr Asp Phe Leu Leu Gln Arg Ile Thr Lys Thr Leu Lys Ser
    450                 455                 460
Asn Ser Trp Phe Gly Cys
465                 470
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 267 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Met Arg Leu Thr Val Leu Cys Ala Val Cys Leu Leu Pro Gly Ser Leu
 1               5                  10                  15
Ala Leu Pro Leu Pro Gln Glu Ala Gly Gly Met Ser Glu Leu Gln Trp
            20                  25                  30
Glu Gln Ala Gln Asp Tyr Leu Lys Arg Phe Tyr Leu Tyr Asp Ser Glu
        35                  40                  45
Thr Lys Asn Ala Asn Ser Leu Glu Ala Lys Leu Lys Glu Met Gln Lys
    50                  55                  60
Phe Phe Gly Leu Pro Ile Thr Gly Met Leu Asn Ser Arg Val Ile Glu
65                  70                  75                  80
Ile Met Gln Lys Pro Arg Cys Gly Val Pro Asp Val Ala Glu Tyr Ser
                85                  90                  95
Leu Phe Pro Asn Ser Pro Lys Trp Thr Ser Lys Val Val Thr Tyr Arg
            100                 105                 110
Ile Val Ser Tyr Thr Arg Asp Leu Pro His Ile Thr Val Asp Arg Leu
        115                 120                 125
Val Ser Lys Ala Leu Asn Met Trp Gly Lys Glu Ile Pro Leu His Phe
    130                 135                 140
Arg Lys Val Val Trp Gly Thr Ala Asp Ile Met Ile Gly Phe Ala Arg
145                 150                 155                 160
Gly Ala His Gly Asp Ser Tyr Pro Phe Asp Gly Pro Gly Asn Thr Leu
                165                 170                 175
Ala His Ala Phe Ala Pro Gly Thr Gly Leu Gly Gly Asp Ala His Phe
            180                 185                 190
Asp Glu Asp Glu Arg Trp Thr Asp Gly Ser Ser Leu Gly Ile Asn Phe
        195                 200                 205
Leu Tyr Ala Ala Thr His Glu Leu Gly His Ser Leu Gly Met Gly His
    210                 215                 220
Ser Ser Asp Pro Asn Ala Val Met Tyr Pro Thr Tyr Gly Asn Gly Asp
225                 230                 235                 240
Pro Gln Asn Phe Lys Leu Ser Gln Asp Asp Ile Lys Gly Ile Gln Lys
```

245 250 255
Leu Tyr Gly Lys Arg Ser Asn Ser Arg Lys Lys
260 265

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 582 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met Ser Pro Ala Pro Arg Pro Ser Arg Cys Leu Leu Leu Pro Leu Leu
1 5 10 15
Thr Leu Gly Thr Ala Leu Ala Ser Leu Gly Ser Ala Gln Ser Ser Ser
20 25 30
Phe Ser Pro Glu Ala Trp Leu Gln Gln Tyr Gly Tyr Leu Pro Pro Gly
35 40 45
Asp Leu Arg Thr His Thr Gln Arg Ser Pro Gln Ser Leu Ser Ala Ala
50 55 60
Ile Ala Ala Met Gln Lys Phe Tyr Gly Leu Gln Val Thr Gly Lys Ala
65 70 75 80
Asp Ala Asp Thr Met Lys Ala Met Arg Arg Pro Arg Cys Gly Val Pro
85 90 95
Asp Lys Phe Gly Ala Glu Ile Lys Ala Asn Val Arg Arg Lys Arg Tyr
100 105 110
Ala Ile Gln Gly Leu Lys Trp Gln His Asn Glu Ile Thr Phe Cys Ile
115 120 125
Gln Asn Tyr Thr Pro Lys Val Gly Glu Tyr Ala Thr Tyr Glu Ala Ile
130 135 140
Arg Lys Ala Phe Arg Val Trp Glu Ser Ala Thr Pro Leu Arg Phe Arg
145 150 155 160
Glu Val Pro Tyr Ala Tyr Ile Arg Glu Gly His Glu Lys Gln Ala Asp
165 170 175
Ile Met Ile Phe Phe Ala Glu Gly Phe His Gly Asp Ser Thr Pro Phe
180 185 190
Asp Gly Glu Gly Gly Phe Leu Ala His Ala Tyr Phe Pro Gly Pro Asn
195 200 205
Ile Gly Gly Asp Thr His Phe Asp Ser Ala Glu Pro Trp Thr Val Arg
210 215 220
Asn Glu Asp Leu Asn Gly Asn Asp Ile Phe Leu Val Ala Val His Glu
225 230 235 240
Leu Gly His Ala Leu Gly Leu Glu His Ser Ser Asp Pro Ser Ala Ile
245 250 255
Met Ala Pro Phe Tyr Gln Trp Met Asp Thr Glu Asn Phe Val Leu Pro
260 265 270
Asp Asp Asp Arg Arg Gly Ile Gln Gln Leu Tyr Gly Gly Glu Ser Gly
275 280 285
Phe Pro Thr Lys Met Pro Pro Gln Pro Arg Thr Thr Ser Arg Pro Ser

NOT FURNISHED UPON FILING

NO PRESENTADO(A) EN EL MOMENTO DE LA PRESENTACIÓN

NON SOUMIS(E) AU MOMENT DU DÉPÔT

```
            20                  25                  30
Leu Leu Val Leu Leu Gly Cys Leu Gly Leu Gly Val Ala Ala Glu Asp
        35                  40                  45
Ala Glu Val His Ala Glu Asn Trp Leu Arg Leu Tyr Gly Tyr Leu Pro
    50                  55                  60
Gln Pro Ser Arg His Met Ser Thr Met Arg Ser Ala Gln Ile Leu Ala
65                  70                  75                  80
Ser Ala Leu Ala Glu Met Gln Arg Phe Tyr Gly Ile Pro Val Thr Gly
                85                  90                  95
Val Leu Asp Glu Glu Thr Lys Glu Trp Met Lys Arg Pro Arg Cys Gly
            100                 105                 110
Val Pro Asp Gln Phe Gly Val Arg Val Lys Ala Asn Leu Arg Arg Arg
        115                 120                 125
Arg Lys Arg Tyr Ala Leu Thr Gly Arg Lys Trp Asn Asn His His Leu
    130                 135                 140
Thr Phe Ser Ile Gln Asn Tyr Thr Glu Lys Leu Gly Trp Tyr His Ser
145                 150                 155                 160
Met Glu Ala Val Arg Arg Ala Phe Arg Val Trp Glu Gln Ala Thr Pro
                165                 170                 175
Leu Val Phe Gln Glu Val Pro Tyr Glu Asp Ile Arg Leu Arg Arg Gln
            180                 185                 190
Lys Glu Ala Asp Ile Met Val Leu Phe Ala Ser Gly Phe His Gly Asp
        195                 200                 205
Ser Ser Pro Phe Asp Gly Thr Gly Gly Phe Leu Ala His Ala Tyr Phe
    210                 215                 220
Pro Gly Pro Gly Leu Gly Gly Asp Thr His Phe Asp Ala Asp Glu Pro
225                 230                 235                 240
Trp Thr Phe Ser Ser Thr Asp Leu His Gly Asn Asn Leu Phe Leu Val
                245                 250                 255
Ala Val His Glu Leu Gly His Ala Leu Gly Leu Glu His Ser Ser Asn
            260                 265                 270
Pro Asn Ala Ile Met Ala Pro Phe Tyr Gln Trp Lys Asp Val Asp Asn
        275                 280                 285
Phe Lys Leu Pro Glu Asp Asp Leu Arg Gly Ile Gln Gln Leu Tyr Gly
    290                 295                 300
Thr Pro Asp Gly Gln Pro Gln Pro Thr Gln Pro Leu Pro Thr Val Thr
305                 310                 315                 320
Pro Arg Arg Pro Gly Arg Pro Asp His Arg Pro Pro Arg Pro Pro Gln
                325                 330                 335
Pro Pro Pro Pro Gly Gly Lys Pro Glu Arg Pro Pro Lys Pro Gly Pro
            340                 345                 350
Pro Val Gln Pro Arg Ala Thr Glu Arg Pro Asp Gln Tyr Gly Pro Asn
        355                 360                 365
Ile Cys Asp Gly Asp Phe Asp Thr Val Ala Met Leu Arg Gly Glu Met
    370                 375                 380
Phe Val Phe Lys Gly Arg Trp Phe Trp Arg Val Arg His Asn Arg Val
385                 390                 395                 400
Leu Asp Asn Tyr Pro Met Pro Ile Gly His Phe Trp Arg Gly Leu Pro
                405                 410                 415
Gly Asp Ile Ser Ala Ala Tyr Glu Arg Gln Asp Gly Arg Phe Val Phe
            420                 425                 430
Phe Lys Gly Asp Arg Tyr Trp Leu Phe Arg Glu Ala Asn Leu Glu Pro
        435                 440                 445
```

```
Gly Tyr Pro Gln Pro Leu Thr Ser Tyr Gly Leu Gly Ile Pro Tyr Asp
    450                 455                 460
Arg Ile Asp Thr Ala Ile Trp Trp Glu Pro Thr Gly His Thr Phe Phe
465                 470                 475                 480
Phe Gln Glu Asp Arg Tyr Trp Arg Phe Asn Glu Glu Thr Gln Arg Gly
                485                 490                 495
Asp Pro Gly Tyr Pro Lys Pro Ile Ser Val Trp Gln Gly Ile Pro Ala
            500                 505                 510
Ser Pro Lys Gly Ala Phe Leu Ser Asn Asp Ala Ala Tyr Thr Tyr Phe
        515                 520                 525
Tyr Lys Gly Thr Lys Tyr Trp Lys Phe Asp Asn Glu Arg Leu Arg Met
    530                 535                 540
Glu Pro Gly Tyr Pro Lys Ser Ile Leu Arg Asp Phe Met Gly Cys Gln
545                 550                 555                 560
Glu His Val Glu Pro Gly Pro Arg Trp Pro Asp Val Ala Arg Pro Pro
                565                 570                 575
Phe Asn Pro His Gly Gly Ala Glu Pro Gly Ala Asp Ser Ala Glu Gly
            580                 585                 590
Asp Val Gly Asp Gly Asp Gly Asp Phe Gly Ala Gly Val Asn Lys Asp
        595                 600                 605
Gly Gly Ser Arg Val Val Val Gln Met Glu Glu Val Ala Arg Thr Val
    610                 615                 620
Asn Val Val Met Val Leu Val Pro Leu Leu Leu Leu Leu Cys Val Leu
625                 630                 635                 640
Gly Leu Thr Tyr Ala Leu Val Gln Met Gln Arg Lys Gly Ala Pro Arg
                645                 650                 655
Val Leu Leu Tyr Cys Lys Arg Ser Leu Gln Glu Trp Val
            660                 665
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 604 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Met Ile Leu Leu Thr Phe Ser Thr Gly Arg Arg Leu Asp Phe Val His
 1               5                  10                  15
His Ser Gly Val Phe Phe Leu Gln Thr Leu Leu Trp Ile Leu Cys Ala
            20                  25                  30
Thr Val Cys Gly Thr Glu Gln Tyr Phe Asn Val Glu Val Trp Leu Gln
        35                  40                  45
Lys Tyr Gly Tyr Leu Pro Pro Thr Ser Pro Arg Met Ser Val Val Arg
    50                  55                  60
Ser Ala Glu Thr Met Gln Ser Ala Leu Ala Ala Met Gln Gln Phe Tyr
65                  70                  75                  80
Gly Ile Asn Met Thr Gly Lys Val Asp Arg Asn Thr Ile Asp Trp Met
                85                  90                  95
```

Lys Lys Pro Arg Cys Gly Val Pro Asp Gln Thr Arg Gly Ser Ser Lys
100 105 110
Phe His Ile Arg Arg Lys Arg Tyr Ala Leu Thr Gly Gln Lys Trp Gln
115 120 125
His Lys His Ile Thr Tyr Ser Ile Lys Asn Val Thr Pro Lys Val Gly
130 135 140
Asp Pro Glu Thr Arg Lys Ala Ile Arg Arg Ala Phe Asp Val Trp Gln
145 150 155 160
Asn Val Thr Pro Leu Thr Phe Glu Glu Val Pro Tyr Ser Glu Leu Glu
165 170 175
Asn Gly Lys Arg Asp Val Asp Ile Pro Ile Ile Phe Ala Ser Gly Phe
180 185 190
His Gly Asp Ser Ser Pro Phe Asp Gly Glu Gly Gly Phe Leu Ala His
195 200 205
Ala Tyr Phe Pro Gly Pro Gly Ile Gly Gly Asp Thr His Phe Asp Ser
210 215 220
Asp Glu Pro Trp Thr Leu Gly Asn Pro Asn His Asp Gly Asn Asp Leu
225 230 235 240
Phe Leu Val Ala Val His Glu Leu Gly His Ala Leu Gly Leu Glu His
245 250 255
Ser Asn Asp Pro Thr Ala Ile Met Ala Pro Phe Tyr Gln Tyr Met Glu
260 265 270
Gln Thr Leu Gln Leu Pro Asn Asp Asp Tyr Arg His Gln Arg Tyr Met
275 280 285
Ser Pro Asp Lys Ile Pro Pro Pro Thr Arg Pro Leu Pro Thr Val Pro
290 295 300
Pro His Arg Ser Ile Pro Pro Ala Asp Pro Arg Lys Asn Asp Arg Pro
305 310 315 320
Lys Pro Pro Arg Pro Pro Thr Gly Arg Pro Ser Tyr Pro Gly Ala Lys
325 330 335
Pro Asn Ile Cys Asp Gly Asn Phe Asn Thr Leu Ala Ile Leu Arg Arg
340 345 350
Glu Met Phe Val Phe Lys Asp Gln Trp Phe Trp Arg Val Arg Asn Asn
355 360 365
Arg Val Met Asp Gly Tyr Pro Met Gln Ile Thr Tyr Phe Trp Arg Gly
370 375 380
Leu Pro Pro Ser Ile Asp Ala Val Tyr Glu Asn Ser Asp Gly Asn Phe
385 390 395 400
Val Phe Phe Lys Gly Asn Lys Tyr Trp Val Phe Lys Asp Thr Thr Leu
405 410 415
Gln Pro Gly Tyr Pro His Asp Leu Ile Thr Leu Gly Ser Gly Ile Pro
420 425 430
Pro His Gly Ile Asp Ser Ala Ile Trp Trp Glu Asp Val Gly Lys Thr
435 440 445
Tyr Phe Phe Lys Gly Asp Arg Tyr Trp Arg Tyr Ser Glu Glu Met Lys
450 455 460
Thr Met Asp Pro Gly Tyr Pro Lys Pro Ile Thr Val Trp Lys Gly Ile
465 470 475 480
Pro Glu Ser Pro Gln Gly Ala Phe Val His Lys Glu Asn Gly Phe Thr
485 490 495
Tyr Phe Tyr Lys Glu Gly Val Leu Glu Ile Gln Thr Thr Arg Tyr Ser
500 505 510
Arg Leu Glu Pro Gly His Pro Arg Ser Ile Leu Lys Asp Leu Ser Gly

```
        515                 520                 525
Cys Asp Gly Pro Thr Asp Arg Val Lys Glu Gly His Ser Pro Pro Asp
    530                 535                 540
Asp Val Asp Ile Val Ile Lys Leu Asp Asn Thr Ala Ser Thr Val Lys
545                 550                 555                 560
Ala Ile Ala Ile Val Ile Pro Cys Ile Leu Ala Leu Cys Leu Leu Val
                565                 570                 575
Leu Val Tyr Thr Val Phe Gln Phe Lys Arg Lys Gly Thr Pro Arg His
            580                 585                 590
Ile Leu Tyr Cys Lys Arg Ser Met Gln Glu Trp Val
        595                 600
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 488 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Met Ala Pro Ala Ala Trp Leu Arg Ser Ala Ala Ala Arg Ala Leu Leu
 1               5                  10                  15
Pro Pro Met Leu Leu Leu Leu Leu Gln Pro Pro Pro Leu Leu Ala Arg
            20                  25                  30
Ala Leu Pro Pro Asp Val His His Leu His Ala Glu Arg Arg Gly Pro
        35                  40                  45
Gln Pro Trp His Ala Ala Leu Pro Ser Ser Pro Ala Pro Ala Pro Ala
    50                  55                  60
Thr Gln Glu Ala Pro Arg Pro Ala Ser Ser Leu Arg Pro Pro Arg Cys
65                  70                  75                  80
Gly Val Pro Asp Pro Ser Asp Gly Leu Ser Ala Arg Asn Arg Gln Lys
                85                  90                  95
Arg Phe Val Leu Ser Gly Gly Arg Trp Glu Lys Thr Asp Leu Thr Tyr
            100                 105                 110
Arg Ile Leu Arg Phe Pro Trp Gln Leu Val Gln Glu Gln Val Arg Gln
        115                 120                 125
Thr Met Ala Glu Ala Leu Lys Val Trp Ser Asp Val Thr Pro Leu Thr
    130                 135                 140
Phe Thr Glu Val His Glu Gly Arg Ala Asp Ile Met Ile Asp Phe Ala
145                 150                 155                 160
Arg Tyr Trp Asp Gly Asp Asp Leu Pro Phe Asp Gly Pro Gly Gly Ile
                165                 170                 175
Leu Ala His Ala Phe Phe Pro Lys Thr His Arg Glu Gly Asp Val His
            180                 185                 190
Phe Asp Tyr Asp Glu Thr Trp Thr Ile Gly Asp Asp Gln Gly Thr Asp
        195                 200                 205
Leu Leu Gln Val Ala Ala His Glu Phe Gly His Val Leu Gly Leu Gln
    210                 215                 220
His Thr Thr Ala Ala Lys Ala Leu Met Ser Ala Phe Tyr Thr Phe Arg
```

```
225                 230                 235                 240
Tyr Pro Leu Ser Leu Ser Pro Asp Asp Cys Arg Gly Val Gln His Leu
                245                 250                 255
Tyr Gly Gln Pro Trp Pro Thr Val Thr Ser Arg Thr Pro Ala Leu Gly
            260                 265                 270
Pro Gln Ala Gly Ile Asp Thr Asn Glu Ile Ala Pro Leu Glu Pro Asp
        275                 280                 285
Ala Pro Pro Asp Ala Cys Glu Ala Ser Phe Asp Ala Val Ser Thr Ile
    290                 295                 300
Arg Gly Glu Leu Phe Phe Phe Lys Ala Gly Phe Val Trp Arg Leu Arg
305                 310                 315                 320
Gly Gly Gln Leu Gln Pro Gly Tyr Pro Ala Leu Ala Ser Arg His Trp
                325                 330                 335
Gln Gly Leu Pro Ser Pro Val Asp Ala Ala Phe Glu Asp Ala Gln Gly
            340                 345                 350
His Ile Trp Phe Phe Gln Gly Ala Gln Tyr Trp Val Tyr Asp Gly Glu
        355                 360                 365
Lys Pro Val Leu Gly Pro Ala Pro Leu Thr Glu Leu Gly Leu Val Arg
    370                 375                 380
Phe Pro Val His Ala Ala Leu Val Trp Gly Pro Glu Lys Asn Lys Ile
385                 390                 395                 400
Tyr Phe Phe Arg Gly Arg Asp Tyr Trp Arg Phe His Pro Ser Thr Arg
                405                 410                 415
Arg Val Asp Ser Pro Val Pro Arg Arg Ala Thr Asp Trp Arg Gly Val
            420                 425                 430
Pro Ser Glu Ile Asp Ala Ala Phe Gln Asp Ala Asp Gly Tyr Ala Tyr
        435                 440                 445
Phe Leu Arg Gly Arg Leu Tyr Trp Lys Phe Asp Pro Val Lys Val Lys
    450                 455                 460
Ala Leu Glu Gly Phe Pro Arg Leu Val Gly Pro Asp Phe Phe Gly Cys
465                 470                 475                 480
Ala Glu Pro Ala Asn Thr Phe Leu
                485
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 517 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Met Gln Gln Phe Gly Gly Leu Glu Ala Thr Gly Ile Asp Glu Ala Thr
 1               5                  10                  15
Leu Ala Leu Met Lys Thr Pro Arg Cys Ser Leu Pro Asp Leu Pro Val
            20                  25                  30
Leu Thr Gln Ala Arg Arg Arg Arg Gln Ala Pro Ala Pro Thr Lys Trp
        35                  40                  45
Asn Lys Arg Asn Leu Ser Trp Arg Val Arg Thr Phe Pro Arg Asp Ser
```

```
    50                  55                  60
Pro Leu Gly His Asp Thr Val Arg Ala Leu Met Tyr Tyr Ala Leu Lys
65                  70                  75                  80
Val Trp Ser Asp Ile Ala Pro Leu Asn Phe His Glu Val Ala Gly Ser
                85                  90                  95
Thr Ala Asp Ile Gln Ile Asp Phe Ser Lys Ala Asp His Asn Asp Gly
            100                 105                 110
Tyr Pro Phe Asp Ala Arg Arg His Arg Ala His Ala Phe Phe Pro Gly
        115                 120                 125
His His His Thr Ala Gly Tyr Thr His Phe Asn Asp Asp Glu Ala Trp
    130                 135                 140
Thr Phe Arg Ser Ser Asp Ala His Gly Met Asp Leu Phe Ala Val Ala
145                 150                 155                 160
Val His Glu Phe Gly His Ala Ile Gly Leu Ser His Val Ala Ala Ala
                165                 170                 175
His Ser Ile Met Arg Pro Tyr Tyr Gln Gly Pro Val Gly Asp Pro Leu
            180                 185                 190
Arg Tyr Gly Leu Pro Tyr Glu Asp Lys Val Arg Val Trp Gln Leu Tyr
        195                 200                 205
Gly Val Arg Glu Ser Val Ser Pro Thr Ala Gln Pro Glu Glu Pro Pro
    210                 215                 220
Leu Leu Pro Glu Pro Pro Asp Asn Arg Ser Ser Ala Pro Pro Arg Lys
225                 230                 235                 240
Asp Val Pro His Arg Cys Ser Thr His Phe Asp Ala Val Ala Gln Ile
                245                 250                 255
Arg Gly Glu Ala Phe Phe Phe Lys Gly Lys Tyr Phe Trp Arg Leu Thr
            260                 265                 270
Arg Asp Arg His Leu Val Ser Leu Gln Pro Ala Gln Met His Arg Phe
        275                 280                 285
Trp Arg Gly Leu Pro Leu His Leu Asp Ser Val Asp Ala Val Tyr Glu
    290                 295                 300
Arg Thr Ser Asp His Lys Ile Val Phe Phe Lys Gly Asp Arg Tyr Trp
305                 310                 315                 320
Val Phe Lys Asp Asn Asn Val Glu Glu Gly Tyr Pro Arg Pro Val Ser
                325                 330                 335
Asp Phe Ser Leu Pro Pro Gly Gly Ile Asp Ala Ala Phe Ser Trp Ala
            340                 345                 350
His Asn Asp Arg Thr Tyr Phe Phe Lys Asp Gln Leu Tyr Trp Arg Tyr
        355                 360                 365
Asp Asp His Thr Arg His Met Asp Pro Gly Tyr Pro Ala Gln Ser Pro
    370                 375                 380
Leu Trp Arg Gly Val Pro Ser Thr Leu Asp Asp Ala Met Arg Trp Ser
385                 390                 395                 400
Asp Gly Ala Ser Tyr Phe Phe Arg Gly Gln Glu Tyr Trp Lys Val Leu
                405                 410                 415
Asp Gly Glu Leu Glu Val Ala Pro Gly Tyr Pro Gln Ser Thr Ala Arg
            420                 425                 430
Asp Trp Leu Val Cys Gly Asp Ser Gln Ala Asp Gly Ser Val Ala Ala
        435                 440                 445
Gly Val Asp Ala Ala Glu Gly Pro Arg Ala Pro Pro Gly Gln His Asp
    450                 455                 460
Gln Ser Arg Ser Glu Asp Gly Tyr Glu Val Cys Ser Cys Thr Ser Gly
465                 470                 475                 480
```

```
Ala Ser Ser Pro Pro Gly Ala Pro Gly Pro Leu Val Ala Ala Thr Met
                485                 490                 495
Leu Leu Leu Leu Pro Pro Leu Ser Pro Gly Ala Leu Trp Thr Ala Ala
            500                 505                 510
Gln Ala Leu Thr Leu
        515
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Arg Xaa Lys Arg
1
```

What is claimed:

1. A purified polynucleotide or fragment thereof derived from human matrix metalloproteinase 19 (MMP19) gene wherein said purified polynucleotide is capable of selectively hybridizing to the nucleic acid of said MMP19 gene, and wherein said purified polynucleotide has at least 50% identity to SEQ ID NO: 1 and fragments, analogs or complements thereof.

2. The purified polynucleotide of Claim 1 wherein said purified polynucleotide is produced by recombinant techniques.

3. The purified polynucleotide of Claim 2 wherein said polynucleotide produced by recombinant techniques comprises a sequence of at least one epitope encoded by MMP19.

4. The purified polynucleotide of Claim 3 wherein said epitope is selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17.

5. A purified polynucleotide of MMP19 which encodes an MMP19 protein comprising an amino acid sequence which has at least 40% identity to SEQ ID NO: 10.

6. The purified polynucleotide of Claim 5 which encodes an MMP19 protein having SEQ ID NO: 10.

7. A recombinant expression system comprising a nucleic acid sequence that encodes an open reading frame derived from MMP19 which is operably linked to a control sequence compatible with a desired host and wherein said nucleic acid sequence is capable of selectively hybridizing to the nucleic acid of said MMP19 gene, and wherein said purified polynucleotide has at least 50% identity to SEQ ID NO: 1 and fragments, analogs or complements thereof.

8. A host cell comprising the recombinant expression system of Claim 7.

9. An MMP19 polypeptide wherein said polypeptide has at least 40% identity to SEQ ID NO: 10 and fragments thereof.

10. The polypeptide of Claim 9 which further possesses the ability to cleave SEQ ID NO: 13.

11. The polypeptide of Claim 9 selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17.

12. A method of detecting the presence of a target polynucleotide of MMP19 in a test sample, comprising:

(a) contacting said test sample with at least one MMP19 specific polynucleotide or complement thereof; and

(b) detecting the presence of said target polynucleotide of MMP19 in the test sample;

wherein said MMP19 specific polynucleotide has at least 50% identity to polynucleotide SEQ ID NO: 1 and fragments, analogs or complements thereof.

13. The method of Claim 14 wherein said target polynucleotide of MMP19 is attached to a solid phase prior to performing step (a).

14. A method for detecting mRNA of MMP19 in a test sample, comprising:

(a) contacting said test sample with at least one primer;

(b) performing reverse transcription in order to produce cDNA;

(c) amplifying said cDNA obtained from step (b) by using other oligonucleotide primer(s) of MMP19 as sense and antisense primer(s) in a first-stage amplification to obtain MMP19 amplicon;

(d) detecting the presence of said MMP19 amplicon in the test sample;

wherein said oligonucleotide primers of MMP19 have at least 50% identity to SEQ ID NO: 1 and fragments, analogs or complements thereof.

15. A method of detecting target MMP19 polynucleotide in a test sample suspected of containing said target, comprising:

(a) contacting said target MMP19 polynucleotide with at lease on MMP19 oligonucleotide as sense primer and with at least one MMP19 oligonucleotide as an antisense primer and amplifying same to obtain a first stage reaction product;

(b) contacting said first stage reaction product with at least one other MMP19 oligonucleotide, with the proviso that the other MMP19 oligonucleotide is located 3' to the

MMP19 oligonucleotides utilized in step (a) and is complementary to said first stage reaction product; and

(c) detecting said target MMP19 polynucleotide,

wherein said MMP19 oligonucleotides utilized in step (a) and step (b) have at least 50% identity to SEQ ID NO: 1 and fragments, analogs or complements thereof.

16. The method of Claim 14 or Claim 15 wherein said test sample is reacted with a solid phase prior to performing step (a) or step (b) or step (c).

17. The method of Claim 14 or Claim 15 wherein said detection step comprises utilizing a detectable label capable of generating a measurable signal.

18. A method for identifying an inhibitor compound of MMP19 comprising the steps of:

(a) providing a reaction mixture comprising

(i) a substrate;

(ii) an MMP19 protein having at least 40% identity to SEQ ID NO: 10 or fragments thereof, said MMP19 protein further possessing the ability to cleave said substrate; and

(iii) a compound of interest;

(b) incubating the reaction mixture; and

(c) determining the extent of cleavage of the substrate in the reaction mixture.

19. The method of Claim 18 wherein said substrate is SEQ ID NO: 13.

20. The method of Claim 19 wherein said MMP19 protein is SEQ ID NO: 10.

21. An antibody which specifically binds to at least one epitope encoded by MMP19, wherein said antibody is polyclonal or monoclonal and wherein said epitope comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17.

22. An assay kit for determining the presence of MMP19 antigen or antibody in a test sample, comprising a container containing an antibody which specifically bins to MMP19 antigen, wherein said antigen comprises at least one epitope of MMP19 having at

least a sequence selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17.

23. The kit of Claim 28 wherein said antibody is attached to a solid phase.

24. A test kit useful for detecting MMP19 polynucleotide in a test sample, comprising a container containing at least one MMP19 polynucleotide which is capable of selectively hybridizing to the nucleic acid of MMP19 gene and wherein said purified polynucleotide has at least 50% identity to SEQ ID NO: 1 an fragments, analogs or complements thereof.

FIG. 1

xxxxxxxxxxxxxxxxxxxLxxLLLLxxxxxxxLxxxxxxxxxxxxxxxxxxxxxxYLxxYYxLx
xxxxxxxxxxxxxxxxxxLxxMQxFFGLxVTGKLDxxTLExMxKPRCGVPDxxxxxxxxxVxx
FxLxPxxPKWxxxxxTYRIxNYTPDLxxxxVDxAIxKAFxVWSxVTPLxFxxVxxxxxxxxxEG
xADIMIxFAxxEHGDxxPFDGPGGxLAHAFxPGPGIGGDAHFDDDExWTxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxGxNLFLVAAHExGHSLGLxHSxDPxAL
MYPxYxxFxDxxxFxLxxDDIxGIQxLYGxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxxxxxxxxxxxPxxCDxxxxxxxxxxxxFDAIxxxRGExFFFKDRFFWRxx
xxxxxxxxxxxLIxxFWPxLPxxIDAAYExxxxxxVFFFKGxxYWxYxGxxxxxGYPxxIxxxL
GFPxxVxxIDAAxxxxxxxxxKTYFFxxxxYWRYDExxxxMDPGYPKxIxxxFxGIxxxVDAVFx
xxxxGFxYFFxGxxxYxFxxxxxxVxxxxxxxxxxxWLxCxxxxxxxxxxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

FIG. 2

```
1                                                 50
         *         *         *         *         *
CCTNCACTNGATTNAGCGATGCCATNAGAGCGTTTNAGTGGGTGTNCCAG
  X  T  X  X  S  D  A  X  R  A  F  X  W  V  X  Q

51                                               100
         *         *         *         *         *
CTACCTGTNAGCGGCGTGTTGGACCGCGCCACCCTGCGCCAGATGANTNG
 L  P  V  S  G  V  L  D  R  A  T  L  R  Q  M  X  X

101                                              150
         *         *         *         *         *
TCCCCGGTGCGGGGTTACAGATACCAACAGTTATGNGGCCTGGGNTGAGA
  P  R  C  G  V  T  D  T  N  S  Y  X  A  W  X  E

151                                              200
         *         *         *         *         *
GGATCAGTGANTTGTTTGCTAGACAACGGACCAAAATGAGGCGTAAGAAA
 R  I  S  X  L  F  A  R  Q  R  T  K  M  R  R  K  K

201                                              250
         *         *         *         *         *
CGNTTTGCAAAGCAAGGTAACAAATGGTANAAGCAGCACCTNTTCTACCG
 R  F  A  K  Q  G  N  K  W  X  K  Q  H  L  F  Y  R

251                                              300
         *         *         *         *         *
NNTGGTGAACTGGGCTGAGNNTGTGCCGGAGCCGGCAGTTGGGGGCGCG
  X  V  N  W  A  E  X  V  P  E  P  A  V  G  G  A
```

FIG. 3

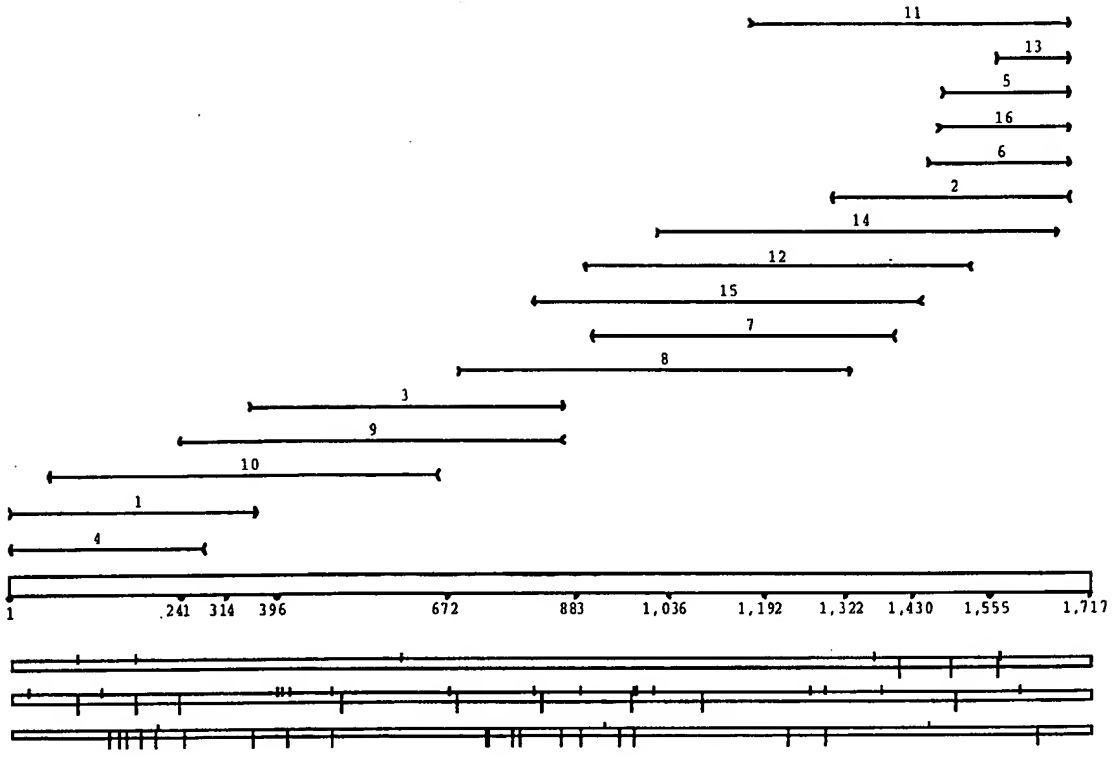

11
13
5
16
6
2
14
12
15
7
8
3
9
10
1
4
1
241
314
396
672
883
1,036
1,192
1,322
1,430
1,555
1,717

FIG. 4(a)

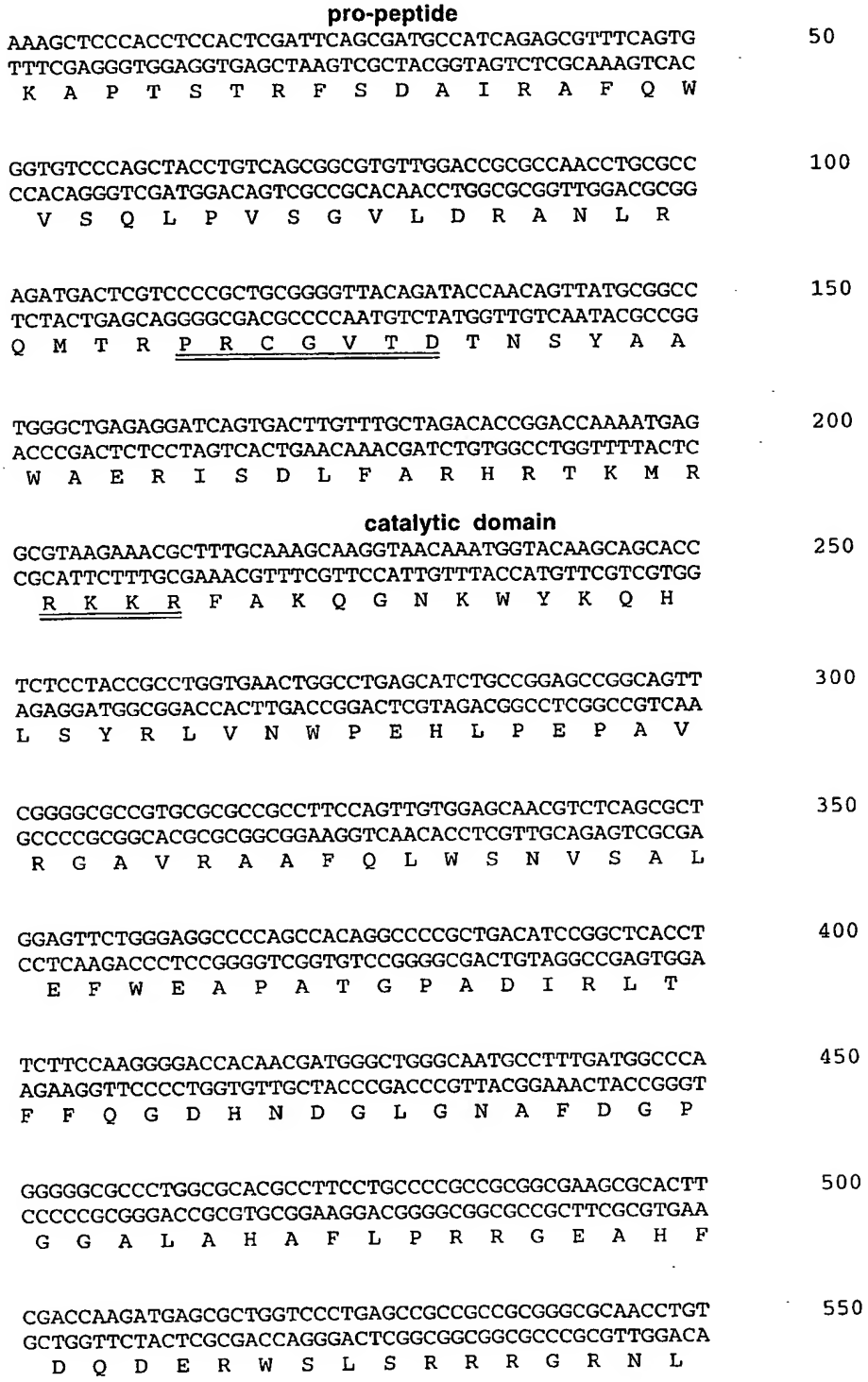

pro-peptide
AAAGCTCCCACCTCCACTCGATTCAGCGATGCCATCAGAGCGTTTCAGTG 50
TTTCGAGGGTGGAGGTGAGCTAAGTCGCTACGGTAGTCTCGCAAAGTCAC
K A P T S T R F S D A I R A F Q W
GGTGTCCCAGCTACCTGTCAGCGGCGTGTTGGACCGCGCCAACCTGCGCC 100
CCACAGGGTCGATGGACAGTCGCCGCACAACCTGGCGCGGTTGGACGCGG
V S Q L P V S G V L D R A N L R
AGATGACTCGTCCCCGCTGCGGGGTTACAGATACCAACAGTTATGCGGCC 150
TCTACTGAGCAGGGGCGACGCCCCAATGTCTATGGTTGTCAATACGCCGG
Q M T R P R C G V T D T N S Y A A
TGGGCTGAGAGGATCAGTGACTTGTTTGCTAGACACCGGACCAAAATGAG 200
ACCCGACTCTCCTAGTCACTGAACAAACGATCTGTGGCCTGGTTTTACTC
W A E R I S D L F A R H R T K M R
catalytic domain
GCGTAAGAAACGCTTTGCAAAGCAAGGTAACAAATGGTACAAGCAGCACC 250
CGCATTCTTTGCGAAACGTTTCGTTCCATTGTTTACCATGTTCGTCGTGG
R K K R F A K Q G N K W Y K Q H
TCTCCTACCGCCTGGTGAACTGGCCTGAGCATCTGCCGGAGCCGGCAGTT 300
AGAGGATGGCGGACCACTTGACCGGACTCGTAGACGGCCTCGGCCGTCAA
L S Y R L V N W P E H L P E P A V
CGGGGCGCCGTGCGCGCCGCCTTCCAGTTGTGGAGCAACGTCTCAGCGCT 350
GCCCCGCGGCACGCGCGGCGGAAGGTCAACACCTCGTTGCAGAGTCGCGA
R G A V R A A F Q L W S N V S A L
GGAGTTCTGGGAGGCCCCAGCCACAGGCCCCGCTGACATCCGGCTCACCT 400
CCTCAAGACCCTCCGGGGTCGGTGTCCGGGGCGACTGTAGGCCGAGTGGA
E F W E A P A T G P A D I R L T
TCTTCCAAGGGGACCACAACGATGGGCTGGGCAATGCCTTTGATGGCCCA 450
AGAAGGTTCCCCTGGTGTTGCTACCCGACCCGTTACGGAAACTACCGGGT
F F Q G D H N D G L G N A F D G P
GGGGGCGCCCTGGCGCACGCCTTCCTGCCCCGCCGCGGCGAAGCGCACTT 500
CCCCCGCGGGACCGCGTGCGGAAGGACGGGGCGGCGCCGCTTCGCGTGAA
G G A L A H A F L P R R G E A H F
CGACCAAGATGAGCGCTGGTCCCTGAGCCGCCGCCGCGGGCGCAACCTGT 550
GCTGGTTCTACTCGCGACCAGGGACTCGGCGGCGGCGCCCGCGTTGGACA
D Q D E R W S L S R R R R G R N L

FIG. 4(b)

```
TCGTGGTGCTGGCGCACGAGATCGGTCACACGCTTGGCCTCACCCACTCG          600
AGCACCACGACCGCGTGCTCTAGCCAGTGTGCGAACCGGAGTGGGTGAGC
F  V  V  L  A  H  E  I  G  H  T  L  G  L  T  H  S

CCCGCGCCGCGCGCGCTCATGGCGCCCTACTACAAGAGGCTGGGCCGCGA          650
GGGCGCGGCGCGCGCGAGTACCGCGGGATGATGTTCTCCGACCCGGCGCT
 P  A  P  R  A  L  M  A  P  Y  Y  K  R  L  G  R  D

CGCGCTGCTCAGCTGGGACGACGTGCTGGCCGTGCAGAGCCTGTATGGGA          700
GCGCGACGAGTCGACCCTGCTGCACGACCGGCACGTCTCGGACATACCCT
  A  L  L  S  W  D  D  V  L  A  V  Q  S  L  Y  G

                                  hinge region
AGCCCCTAGGGGGCTCAGTGGCCGTCCAGCTCCCAGGAAAGCTGTTCACT          750
TCGGGGATCCCCCGAGTCACCGGCAGGTCGAGGGTCCTTTCGACAAGTGA
K  P  L  G  G  S  V  A  V  Q  L  P  G  K  L  F  T

GACTTTGAGACCTGGGACTCCTACAGCCCCCAAGGAAGGCGCCCTGAAAC          800
CTGAAACTCTGGACCCTGAGGATGTCGGGGGTTCCTTCCGCGGGACTTTG
 D  F  E  T  W  D  S  Y  S  P  Q  G  R  R  P  E  T

                                  hemopexin domain
GCAGGGCCCTAAATACTGCCACTCTTCCTTCGATGCCATCACTGTAGACA          850
CGTCCCGGGATTTATGACGGTGAGAAGGAAGCTACGGTAGTGACATCTGT
  Q  G  P  K  Y  C  H  S  S  F  D  A  I  T  V  D

GGCAACAGCAACTGTACATTTTTAAAGGGAGCCATTTCTGGGAGGTGGCA          900
CCGTTGTCGTTGACATGTAAAAATTTCCCTCGGTAAAGACCCTCCACCGT
R  Q  Q  Q  L  Y  I  F  K  G  S  H  F  W  E  V  A

GCTGATGGCAACGTCTCAGAGCCCCGTCCACTGCAGGAAAGATGGGTCGG          950
CGACTACCGTTGCAGAGTCTCGGGGCAGGTGACGTCCTTTCTACCCAGCC
 A  D  G  N  V  S  E  P  R  P  L  Q  E  R  W  V  G

GCTGCCCCCCAACATTGAGGCTGCGGCAGTGTCATTGAATGATGGAGATT         1000
CGACGGGGGGTTGTAACTCCGACGCCGTCACAGTAACTTACTACCTCTAA
  L  P  P  N  I  E  A  A  A  V  S  L  N  D  G  D

TCTACTTCTTCAAAGGGGGTCGATGCTGGAGGTTCCGGGGCCCCAAGCCA         1050
AGATGAAGAAGTTTCCCCCAGCTACGACCTCCAAGGCCCCGGGGTTCGGT
F  Y  F  F  K  G  G  R  C  W  R  F  R  G  P  K  P

GTGTGGGGTCTCCCACAGCTGTGCCGGGCAGGGGGCCTGCCCCGCCATCC         1100
CACACCCCAGAGGGTGTCGACACGGCCCGTCCCCCGGACGGGGCGGTAGG
 V  W  G  L  P  Q  L  C  R  A  G  G  L  P  R  H  P

TGACGCCGCCCTCTTCTTCCCTCCTCTGCGCCGCCTCATCCTCTTCAAGG         1150
ACTGCGGCGGGAGAAGAAGGGAGGAGACGCGGCGGAGTAGGAGAAGTTCC
  D  A  A  L  F  F  P  P  L  R  R  L  I  L  F  K
```

FIG. 4(c)

```
GTGCCCGCTACTACGTGCTGGCCCGAGGGGGACTGCAAGTGGAGCCCTAC          1200
CACGGGCGATGATGCACGACCGGGCTCCCCCTGACGTTCACCTCGGGATG
G  A  R  Y  Y  V  L  A  R  G  G  L  Q  V  E  P  Y

TACCCCCGAAGTCTGCAGGACTGGGGAGGCATCCCTGAGGAGGTCAGCGG          1250
ATGGGGGCTTCAGACGTCCTGACCCCTCCGTAGGGACTCCTCCAGTCGCC
 Y  P  R  S  L  Q  D  W  G  G  I  P  E  E  V  S  G

CGCCCTGCCGAGGCCCGATGGCTCCATCATCTTCTTCCGAGATGACCGCT          1300
GCGGGACGGCTCCGGGCTACCGAGGTAGTAGAAGAAGGCTCTACTGGCGA
  A  L  P  R  P  D  G  S  I  I  F  F  R  D  D  R

ACTGGCGCCTCGACCAGGCCAAACTGCAGGCAACCACCTCGGGCCGCTGG          1350
TGACCGCGGAGCTGGTCCGGTTTGACGTCCGTTGGTGGAGCCCGGCGACC
Y  W  R  L  D  Q  A  K  L  Q  A  T  T  S  G  R  W

GCCACCGAGCTGCCCTGGATGGGCTGCTGGCATGCCAACTCGGGGAGCGC          1400
CGGTGGCTCGACGGGACCTACCCGACGACCGTACGGTTGAGCCCCTCGCG
 A  T  E  L  P  W  M  G  C  W  H  A  N  S  G  S  A

CCTGTTCTGAAGGCACCTCCTCACCTCAGAAACTGGTGGTGCTCTCAGGG          1450
GGACAAGACTTCCGTGGAGGAGTGGAGTCTTTGACCACCACGAGAGTCCC
  L  F  *

CAAAATCATGTTCCCCACCCCCGGGGCAGAACCCCTCTTAGAAGCCTCTG          1500
GTTTTAGTACAAGGGGTGGGGGCCCCGTCTTGGGGAGAATCTTCGGAGAC

AGTCCCTCTGCAGAAGACCGGGCAGCAAAGCCTCCATCTGGAAGTCTGTC          1550
TCAGGGAGACGTCTTCTGGCCCGTCGTTTCGGAGGTAGACCTTCAGACAG

TGCCTTTGTTCCTTGAAGAATGCAGCATTGTCTTTGTCTGTCCCCACCAC          1600
ACGGAAACAAGGAACTTCTTACGTCGTAACAGAAACAGACAGGGGTGGTG

ATGGAGGTGGGGGTGGGATCAATCTTAGGAAAAGCAAAAAAGGGTCCCAG          1650
TACCTCCACCCCCACCCTAGTTAGAATCCTTTTCGTTTTTTCCCAGGGTC

ATCCCTTGGCCCTTTCCTCCGAAAAAAAAAAAAAAAAAAAAAAAAAAAA          1700
TAGGGAACCGGGAAAGGAGGCTTTTTTTTTTTTTTTTTTTTTTTTTTTT

AAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTTT
```

FIG. 5(a)

```
                1                                                   50
MMP19           .......... .......... .......... .......... ..........
Matrilysin      .......... .......... ...MRLTVLC AVCL.LPGSL ALPLPQEAGG
Gelatinase A    .......... ..MEALMARG ALTGPLRALC LLGCLLSHA. AAAPSPIIKF
Gelatinase B    .......... .....MSLWQ PL...VLVLL VLGCCFAAP. RQRQSTLVLF
Stromelysin 1   .......... .......... ..MKSLPILL LLCVAVCSAY PLDGAARGED
Stromelysin 2   .......... .......... ..MMHLAFLV LLCLPVCSAY PLSGAAKEED
Stromelysin 3   .......... MAPAAWLRSA AARALLPPML LLLLQPPPLL ARALPPDVHH
Collagenase 1   .......... .........M HSFPPLLLLL FWGV.VSHSF PATLETQEQD
Collagenase 2   .......... .........M FSLKTLPFLL LLHVQISKAF P..VSSKEKN
Collagenase 3   .......... .......... MHPGVLAAFL FLSWTHCRAL PLPSGGDEDD
MMP12           .......... .......... ...MKFLLIL LLQATASGAL PLNSSTSLEK
MMP18           .......... .......... .MNCQQLWLG FLLPMTVSGR VLGLAEVAPV
mt1MMP          ....MSPAPR PSRC...... ........LL LPLLTLGTAL ASLGSAQSSS
mt2MMP          MGSDPSAPGR PGWTGSLLGD REEAARPRLL PLLLVLLGCL GLGVAAEDAE
mt3MMP          .......... MILLTFSTGR RLDFVHHSGV FFLQTLLWIL CATVCGTEQY
mt4MMP          .......... .......... .......... .......... ..........

Consensus       ---------- ---------- -----L--LL LLLL-L--AL PL--S--E--


                51                                                  100
MMP19           .......... .......... ........KA PTSTRFSDAI RAFQWVSQLP
Matrilysin      MSELQWEQAQ DYLKRFYLYD SETKN..... ..ANSLEAKL KEMQKFFGLP
Gelatinase A    PGDVAPK.TD KELAVQYL.. NTFYGCPKES CNLFVLKDTL KKMQKFFGLP
Gelatinase B    PGDLRTNLTD RQLAEEYLYR YGYTRVAEMR GESKSLGPAL LLLQKQLSLP
Stromelysin 1   TS...MNLVQ KYLENYYDLK KDVKQFVRRK DSGPVV.KKI REMQKFLGLE
Stromelysin 2   SN...KDLAQ QYLEKYYNLE KDVKQF.RRK DSNLIV.KKI QGMQKFLGLE
Stromelysin 3   LH......AE RR........ GPQPWHAALP .SSPAPAPAT QEAPR.....
Collegenase 1   V.....DLVQ KYLEKYYNLK NDGRQVEKRR NSGPVV.EKL KQMQEFFGLK
Collegenase 2   T.....KTVQ DYLEKFYQLP SNQYQSTRKN GTNVIV.EKL KEMQRFFGLN
Collegenase 3   LSEEDLQFAE RYLRSYYH.P TNLAGILKEN AASSMT.ERL REMQSFFGLE
MMP12           NNVL...FGE RYLEKFYGLE INKLPVTKMK YSGNLMKEKI QEMQHFLGLK
MMP18           DYLSQYGYLQ KPLE...... ......GSNN FKPEDITEAL RAFQEASELP
mt1MMP          FS......PE AWLQQYGYLP PGDLRTHTQR .SPQSLSAAI AAMQKFYGLQ
mt2MMP          VH......AE NWLRLYGYLP QPSRHMSTMR .SAQILASAL AEMQRFYGIP
mt3MMP          FN......VE VWLQKYGYLP PTSPRMSVVR .SAETMQSAL AAMQQFYGIN
mt4MMP          .......... .......... .......... .......... ..MQQFGGLE

Consensus       --------A- -YLEKYY-LP ---------R -S---L-EAL -EMQKFFGLP
```

FIG. 5(b)

```
                 101                                                 150
MMP19            VSGVLDRANL RQMTRPRCGV TDTNSYAAWA ERISDLFARH RTKMRRKKRF
Matrilysin       ITGMLNSRVI EIMQKPRCGV PDVAEYSLFP N..........  ..........
Gelatinase A     QTGDLDQNTI ETMRKPRCGN PDVANYNFFP R..........  ..........
Gelatinase B     ETGELDSATL KAMRTPRCGV PDLGRFQTFE G..........  ..........
Stromelysin 1    VTGKLDSDTL EVMRKPRCGV PDVGHFRTFP G..........  ..........
Stromelysin 2    VTGKLDTDTL EVMRKPRCGV PDVGHFSSFP G..........  ..........
Stromelysin 3    ........PA SSLRPPRCGV PDPSDGLSAR N..........  .....RQKRF
Collegenase 1    VTGKPDAETL KVMKQPRCGV PDVAQFVLTE G..........  ..........
Collegenase 2    VTGKPNEETL DMMKKPRCGV PDSGGFMLTP G..........  ..........
Collegenase 3    VTGKLDDNTL DVMKKPRCGV PDVGEYNVFP R..........  ..........
MMP12            VTGQLDTSTL EMMHAPRCGV PDVHHFREMP G..........  ..........
MMP18            VSGQLDDATR ARMRQPRCGL ED.....PFN QKTLKYLLLG R...WRKK..
mt1MMP           VTGKADADTM KAMRRPRCGV PDKFGAEIKA N..........  ..V..RRKRY
mt2MMP           VTGVLDEETK EWMKRPRCGV PDQFGVRVKA N..........  ..LRRRRKRY
mt3MMP           MTGKVDRNTI DWMKKPRCGV PDQ..TRGSS K..........  ..FHIRRKRY
mt4MMP           ATG.IDEATL ALMKTPRCSL PDLPVLT...  ..........  ...QARRRRQ

Consensus        VTGKLD--TL E-MRKPRCGV PDVG-F--FP G----------  -----R-KR-

                 151                                                 200
MMP19            AKQGNKWYKQ HLSYRLVNWP EH..LPEPAV RGAVRAAFQL WSNVSALEFW
Matrilysin       ...SPKWTSK VVTYRIVSYT RD..LPHITV DRLVSKALNM WGKEIPLHFR
Gelatinase A     ...KPKWDKN QITYRIIGYT PD..LDPETV DDAFARAFQV WSDVTPLRFS
Gelatinase B     ...DLKWHHH NITYWIQNYS ED..LPRAVI DDAFARAFAL WSAVTPLTFT
Stromelysin 1    ...IPKWRKT HLTYRIVNYT PD..LPKDAV DSAVEKALKV WEEVTPLTFS
Stromelysin 2    ...MPKWRKT HLTYRIVNYT PD..LPRDAV DSAIEKALKV WEEVTPLTFS
Stromelysin 3    VLSGGRWEKT DLTYRILRFP WQ..LVQEQV RQTMAEALKV WSDVTPLTFT
Collegenase 1    ...NPRWEQT HLTYRIENYT PD..LPRADV DHAIEKAFQL WSNVTPLTFT
Collegenase 2    ...NPKWERT NLTYRIRNYT PQ..LSEAEV ERAIKDAFEL WSVASPLIFT
Collegenase 3    ...TLKWSKM NLTYRIVNYT PD..MTHSEV EKAFKKAFKV WSDVTPLNFT
MMP12            ...GPVWRKH YITYRINNYT PD..MNREDV DYAIRKAFQV WSNVTPLKFS
MMP18            .......... HLTFRILNLP ST..LPPHTA RAALRQAFQD WSNVAPLTFQ
mt1MMP           AIQGLKWQHN EITFCIQNYT PK..VGEYAT YEAIRKAFRV WESATPLRFR
mt2MMP           ALTGRKWNNH HLTFSIQNYT EK..LGWYHS MEAVRRAFRV WEQATPLVFQ
mt3MMP           ALTGQKWQHK HITYSIKNVT PK..VGDPET RKAIRRAFDV WQNVTPLTFE
mt4MMP           APAPTKWNKR. NLSWRVRTFP RDSPLGHDTV RALMYYALKV WSDIAPLNFH

Consensus        A--GPKW-KT HLTYRIVNYT PD--LP---V D-AIRKAF-V WSNVTPLTFT
```

FIG. 5(c)

```
                201                                                 250
MMP19           EAP..... ATGPADIRLT FFQGDHNDGL GNAFDGPGGA LAHAFLPR.R
Matrilysin      KV..... VWGTADIMIG FARGAHGDSY ..PFDGPGNT LAHAFAPG.T
Gelatinase A    RI..... HDGEADIMIN FGRWEHGDGY ..PFDGKDGL LAHAFAPG.T
Gelatinase B    RV..... YSRDADIVIQ FGVAEHGDGY ..PFDGKDGL LAHAFPPG.P
Stromelysin 1   RL..... YEGEADIMIS FAVREHGDFY ..PFDGPGNV LAHAYAPG.P
Stromelysin 2   RL..... YEGEADIMIS FAVKEHGDFY ..SFDGPGHS LAHAYPPG.P
Stromelysin 3   EV..... HEGRADIMID FARYWDGDDL ..PFDGPGGI LAHAFFPK.T
Collegenase 1   KV..... SEGQADIMIS FVRGDHRDNS ..PFDGPGGN LAHAFQPG.P
Collegenase 2   RI..... SQGEADINIA FYQRDHGDNS ..PFDGPNGI LAHAFQPG.Q
Collegenase 3   RL..... HDGIADIMIS FGIKEHGDFY ..PFDGPSGL LAHAFPPG.P
MMP12           KI..... NTGMADILVV FARGAHGDFH ..AFDGKGGI LAHAFGPG.S
MMP18           EVQ..... A.GAADIRLS .FHGRQSSYC SNTFDGPGRV LAHADIPE.L
mt1MMP          EVPYAYIREG HEKQADIMIF FAEGFHGDST ..PFDGEGGF LAHAYFPG.P
mt2MMP          EVPYEDIRLR RQKEADIMVL FASGFHGDSS ..PFDGTGGF LAHAYFPG.P
mt3MMP          EVPYSELENG K.RDVDIPII FASGFHGDSS ..PFDGEGGF LAHAYFPG.P
mt4MMP          EV..... AGSTADIQID FSKADHNDGY ..PFDARRHR .AHAFFPGHH

Consensus       EV-------- -EGEADIMIS FARGEHGD-Y --PFDGPGG- LAHAFFPG-P


                251                                                 300
MMP19           ...GEAHFDQ DERWSLSR.. .......... .......... ..........
Matrilysin      GLGGDAHFDE DERWTDGSSL .......... .......... ..........
Gelatinase A    GVGGDSHFDD DELWTLGEGQ VVRVKYGNAD GEYCKFPFLF NGKEYNSCTD
Gelatinase B    GIQGDAHFDD DELWSLGKGV VVPTRFGNAD GAACHFPFIF EGRSYSACTT
Stromelysin 1   GINGDAHFDD DEQWTKDTT. .......... .......... ..........
Stromelysin 2   GLYGDIHFDD DEKWTEDAS. .......... .......... ..........
Stromelysin 3   HREGDVHFDY DETWTIG... .......... .......... ..........
Collegenase 1   GIGGDAHFDE DERWTNNFR. .......... .......... ..........
Collegenase 2   GIGGDAHFDA EETWTNTSA. .......... .......... ..........
Collegenase 3   NYGGDAHFDD DETWTSSSK. .......... .......... ..........
MMP12           GIGGDAHFDE DEFWTTHSG. .......... .......... ..........
MMP18           ...GSVHFDE DEFWTEGT.. .......... .......... ..........
mt1MMP          NIGGDTHFDS AEPWTVRNE. .......... .......... ..........
mt2MMP          GLGGDTHFDA DEPWTFSST. .......... .......... ..........
mt3MMP          GIGGDTHFDS DEPWTLGNP. .......... .......... ..........
mt4MMP          HTAGYTHFND DEAWTFRSS. .......... .......... ..........

Consensus       GIGGDAHFDD DE-WT-GS-- ---------- ---------- ----------
```

FIG. 5(d)

```
                301                                                       350
MMP19           .......... .......... .......... .......... ..........
Matrilysin      .......... .......... .......... .......... ..........
Gelatinase A    TGRSDGFLWC STTYNFEKDG KYGFCPHEAL FTMGGNAEGQ PCKFPFRFQG
Gelatinase B    DGRSDGLPWC STTANYDTDD RFGFCPSERL YTRDGNADGK PCQFPFIFQG
Stromelysin 1   .......... .......... .......... .......... ..........
Stromelysin 2   .......... .......... .......... .......... ..........
Stromelysin 3   .......... .......... .......... .......... ..........
Collegenase 1   .......... .......... .......... .......... ..........
Collegenase 2   .......... .......... .......... .......... ..........
Collegenase 3   .......... .......... .......... .......... ..........
MMP12           .......... .......... .......... .......... ..........
MMP18           .......... .......... .......... .......... ..........
mt1MMP          .......... .......... .......... .......... ..........
mt2MMP          .......... .......... .......... .......... ..........
mt3MMP          .......... .......... .......... .......... ..........
mt4MMP          .......... .......... .......... .......... ..........

Consensus       ---------- ---------- ---------- ---------- ----------


                351                                                       400
MMP19           .......... .......... .......... .......... ..........
Matrilysin      .......... .......... .......... .......... ..........
Gelatinase A    TSYDSCTTEG RTDGYRWCGT TEDYDRDKKY GFCPETAMST V.GGNSEGAP
Gelatinase B    QSYSACTTDG RSDGYRWCAT TANYDRDKLF GFCPTRADST VMGGNSAGEL
Stromelysin 1   .......... .......... .......... .......... ..........
Stromelysin 2   .......... .......... .......... .......... ..........
Stromelysin 3   .......... .......... .......... .......... ..........
Collegenase 1   .......... .......... .......... .......... ..........
Collegenase 2   .......... .......... .......... .......... ..........
Collegenase 3   .......... .......... .......... .......... ..........
MMP12           .......... .......... .......... .......... ..........
MMP18           .......... .......... .......... .......... ..........
mt1MMP          .......... .......... .......... .......... ..........
mt2MMP          .......... .......... .......... .......... ..........
mt3MMP          .......... .......... .......... .......... ..........
mt4MMP          .......... .......... .......... .......... ..........

Consensus       ---------- ---------- ---------- ---------- ----------
```

FIG. 5(e)

```
                401                                                 450
MMP19           .......... .......... .......... .......... ...RRGRNLF
Matrilysin      .......... .......... .......... .......... .....GINFL
Gelatinase A    CVFPFTFLGN KYESCTSAGR SDGKMWCATT ANYDDDRKWG FCPDQGYSLF
Gelatinase B    CVFPFTFLGK EYSTCTSEGR GDGRLWCATT SNFDSDKKWG FCPDQGYSLF
Stromelysin 1   .......... .......... .......... .......... .....GTNLF
Stromelysin 2   .......... .......... .......... .......... .....GTNLF
Stromelysin 3   .......... .......... .......... .......... ..DDQGTDLL
Collegenase 1   .......... .......... .......... .......... .....EYNLH
Collegenase 2   .......... .......... .......... .......... .....NYNLF
Collegenase 3   .......... .......... .......... .......... .....GYNLF
MMP12           .......... .......... .......... .......... .....GTNLF
MMP18           .......... .......... .......... .......... ...YRGVNLR
mt1MMP          .......... .......... .......... .......... ..DLNGNDIF
mt2MMP          .......... .......... .......... .......... ..DLHGNNLF
mt3MMP          .......... .......... .......... .......... ..NHDGNDLF
mt4MMP          .......... .......... .......... .......... ..DAHGMDLF

Consensus       ---------- ---------- ---------- ---------- -----GYNLF


                451                                                 500
MMP19           VVLAHEIGHT LGLTHSPAPR ALMAPYYKR. LG..RDALLS WDDVLAVQSL
Matrilysin      YAATHELGHS LGMGHSSDPN AVMYPTYGN. .GDPQNFKLS QDDIKGIQKL
Gelatinase A    LVAAHEFGHA MGLEHSQDPG ALMAPIY... .TYTKNFRLS QDDIKGIQEL
Gelatinase B    LVAAHEFGHA LGLDHSSVPE ALMYPMY... .RFTEGPPLH KDDVNGIRHL
Stromelysin 1   LVAAHEIGHS LGLFHSANTE ALMYPLYHS. LTDLTRFRLS QDDINGIQSL
Stromelysin 2   LVAAHELGHS LGLFHSANTE ALMYPLYNS. FTELAQFRLS QDDVNGIQSL
Stromelysin 3   QVAAHEFGHV LGLQHTTAAK ALMSAFY.T. FRYP..LSLS PDDCRGVQHL
Collegenase 1   RVAAHELGHS LGLSHSTDIG ALMYPSY.T. F..SGDVQLA QDDIDGIQAI
Collegenase 2   LVAAHEFGHS LGLAHSSDPG ALMYPNY.A. FRETSNYSLP QDDIDGIQAI
Collegenase 3   LVAAHEFGHS LGLDHSKDPG ALMFPIY.T. YTGKSHFMLP DDDVQGIQSL
MMP12           LTAVHEIGHS LGLGHSSDPK AVMFPTY.K. YVDINTFRLS ADDIRGIQSL
MMP18           IIAAHEVGHA LGLGHSRYSQ ALMAPVYEG. YR..PHFKLH PDDVAGIQAL
mt1MMP          LVAVHELGHA LGLEHSSDPS AIMAPFY.Q. WMDTENFVLP DDDRRGIQQL
mt2MMP          LVAVHELGHA LGLEHSSNPN AIMAPFY.Q. WKDVDNFKLP EDDLRGIQQL
mt3MMP          LVAVHELGHA LGLEHSNDPT AIMAPFY.Q. YME.QTLQLP NDDYR..HQR
mt4MMP          AVAVHEFGHA IGLSHVAAAH SIMRPYYQGP VGDPLRYGLP YEDKVRVWQL

Consensus       LVAAHE-GH- LGL-HSSDP- ALM-P-Y--- --D--NF-LS QDDIRGIQSL
```

FIG. 5(f)

	501			550
MMP19	YGKPLGGSVA VQLPGKLF..	.....	.....	.....
Matrilysin	YGKR...SNS RKK*.....	.....	.....	.....
Gelatinase A	YGAS...PDI D.....	.....	.....	.....
Gelatinase B	YGPR...PEP EPRPPTTTTP	QPTAPPTVCP	TGPPTVHPSE	RPTAGPTGPP
Stromelysin 1	YGPP...PDS PETP.....	.....	.....	.....
Stromelysin 2	YGPP...PAS TEEP.....	.....	.....	.....
Stromelysin 3	YG..... .QPWPTVTSR	.....	....TPALGP	QAGIDTNEIA
Collegenase 1	YGRS...QNP VQ.....	.....	.....	.....
Collegenase 2	YGLS...SNP IQ.....	.....	.....	.....
Collegenase 3	YGPG...DED PN.....	.....	.....	.....
MMP12	YGDP...KEN QRLP.....	.....	.....	.....
MMP18	YGKK...SPV IRDEEEEE..	.....	.....	.....
mt1MMP	YGGESGFPTK MPPQPRTTSR	.....	.....PSVPD	KPKNPT....
mt2MMP	YGTPDGQPQP TQPLPTVTPR	RPGRPDHRPP	RPPQPPPPGG	KPERPPKPGP
mt3MMP	YMSPDKIPPP TRPLPTVPPH	R.....	.....SIPPA	DPRKNDRPKP
mt4MMP	YGVRESVSPT AQP.....	.....	.....	.....
Consensus	YG-P---P-P -QPPP-----	----------	----------	----------

	551			600
MMP19	....TDFETW DSYSPQGRRP	ETQGPKYCHS	SFDAITVDRQ	QQLYIFKGSH
Matrilysin	.....	.....	.....	.....
Gelatinase A	.LGTGPTPTL GPVTPEICKQ	DIV.....	.FDGIAQ.IR	GEIFFFKDRF
Gelatinase B	SAGPTGPPTA GPSTATTVPL	SPVDDACNVN	IFDAIAE.IG	NQLYLFKDGK
Stromelysin 1	.LVPTEPVPP EPGTPANCDP	AL.....	SFDAVST.LR	GEILIFKDRH
Stromelysin 2	.LVPTKSVPS GSEMPAKCDP	AL.....	SFDAIST.LR	GEYLFFKDRY
Stromelysin 3	PLEPDA.... ...PPDACEA	S.....	.FDAVST.IR	GELFFFKAGF
Collegenase 1	.....PI GPQTPKACDS	KL.....	TFDAITT.IR	GEVMFFKDRF
Collegenase 2	.....PT GPSTPKPCDP	SL.....	TFDAITT.LR	GEILFFKDRY
Collegenase 3	.....PK HPKTPDKCDP	SL.....	SLDAITS.LR	GETMIFKDRF
MMP12	.....NP DNSEPALCDP	NL.....	SFDAVTT.VG	NKIFFFKDRF
MMP18	....TELPT. ...VPPVPTE	PSPMPDPCSS	ELDAMMLGPR	GKTYAFKGDY
mt1MMP	....YGPNICDG	N.....	.FDTVAM.LR	GEMFVFKKRW
mt2MMP	PVQPRATERP DQYGPNICDG	D.....	.FDTVAM.LR	GEMFVFKGRW
mt3MMP	PRPPTGRPSY PGAKPNICDG	N.....	.FNTLAI.LR	REMFVFKDQW
mt4MMP	..EEPPLLPE PPDNRSSAPP	RKDVPHRCST	HFDAVA.QIR	GEAFFFKGKY
Consensus	---PT---P- GP-TP--CDP	-L--------	SFDAIAT-LR	GE-FFFKDRF

FIG. 5(g)

	601			650
MMP19	FWEVAADGNV SEPRP..LQE	RWVGLP...P	NIEAAAVSLN	DGDFYFFKGG
Matrilysin	....	.....	.....	.....
Gelatinase A	IWRTVTPRD. KPMGPLLVAT	FWPELP...E	KIDAVYEAPQ	EEKAVFFAGN
Gelatinase B	YWRFSEGRGS RPQGPFLIAD	KWPALP...R	KLDSVFEEPL	SKKLFFFSGR
Stromelysin 1	FWRKSLRKLE PELH..LISS	FWPSLP...S	GVDAAYEVTS	KDLVFIFKGN
Stromelysin 2	FWRRSHWNPE PEFH..LISA	FWPSLP...S	YLDAAYEVNS	RDTVFIFKGN
Stromelysin 3	VWRLRGGQLQ PGY.PALASR	HWQGLP...S	PVDAAFE.DA	QGHIWFFQGA
Collegenase 1	YMRTNPFYPE VELN..FISV	FWPQLP...N	GLEAAYEFAD	RDEVRFFKGN
Collegenase 2	FWRRHPQLQR VEMN..FISL	FWPSLP...T	GIQAAYEDFD	RDLIFLFKGN
Collegenase 3	FWRLHPQQVD AELF..LTKS	FWPELP...N	RIDAAYEHPS	HDLIFIFRGR
MMP12	FWLKVSERPK TSVN..LISS	LWPTLP...S	GIEAAYEIEA	RNQVFLFKDD
MMP18	VWTVSDSGPG PLFR...VSA	LWEGLP...G	NLDAAVYSPR	TQWIHFFKGD
mt1MMP	FWRVRNNQVM DGY.PMPIGQ	FWRGLP...A	SINTAYE.RK	DGKFVFFKGD
mt2MMP	FWRVRHNRVL DNY.PMPIGH	FWRGLP...G	DISAAYE.RQ	DGRFVFFKGD
mt3MMP	FWRVRNNRVM DGY.PMQITY	FWRGLP...P	SIDAVYE.NS	DGNFVFFKGN
mt4MMP	FWRLTRDRHL VSLQPAQMHR	FWRGLPLHLD	SVDAVYERTS	DHKIVFFKGD
Consensus	FWRV---R-- -E--P-LIS-	FWPGLP----	-IDAAYE--S	D--IFFFKGN

	651			700
MMP19	RCWRFRGPKP VWGLPQLCR.	...AGGLPRH	PDAALFF.PP	LRRLILFKGA
Matrilysin	....	.....	.....	.....
Gelatinase A	EYWIY.SAST LERGYPKPLT	SLGLPPDVQR	VDAAF.NWSK	NKKTYIFAGD
Gelatinase B	QVWVYTGASV LG...PRRLD	KLGLGADVAQ	VTGAL..RSG	RGKMLLFSGR
Stromelysin 1	QFWAIRGNEV RAGYPRGIHT	.LGFPPTVRK	IDAAI.SDKE	KNKTYFFVED
Stromelysin 2	EFWAIRGNEV QAGYPRGIHT	.LGFPPTIRK	IDAAV.SDKE	KKKTYFFAAD
Stromelysin 3	QYWVYDGEKP VLG.PAPL.T	EL..GLVRFP	VHAALVWGPE	KNKIYFFRGR
Collegenase 1	KYWAVQGQNV LHGYPKDIYS	SFGFPRTVKH	IDAAL.SEEN	TGKTYFFVAN
Collegenase 2	QYWALSGYDI LQGYPKDI.S	NYGFPSSVQA	IDAAV.F..Y	RSKTYFFVND
Collegenase 3	KFWALNGYDI LEGYPKKI.S	ELGLPKEVKK	ISAAV.HFED	TGKTLLFSGN
MMP12	KYWLISNLRP EPNYPKSIHS	.FGFPNFVKK	IDAAV.FNPR	FYRTYFFVDN
MMP18	KVWRYINFKM SPGFPK..K.	...LNRSEPN	LDAALYW.PL	NQKVFLFKGS
mt1MMP	KHWVFDEASL EPGYPKHI.K	ELGRGLPTDK	IDAALFWMPN	.GKTYFFRGN
mt2MMP	RYWLFREANL EPGYPQPL.T	SYGLGIPYDR	IDTAIWWEPT	.GHTFFFQED
mt3MMP	KYWVFKDTTL QPGYPHDL.I	TLGSGIPPHG	IDSAIWWEDV	.GKTYFFKGD
mt4MMP	RYWVFKDNNV EEGYPRPVS.	..DFSLPPGG	IDAAFSW.AH	NDRTYFFKDQ
Consensus	KYW-F-G--V LPGYPK-I-T	-LGFP--V-K	IDAAL-W-P-	-GKTYFF-GD

FIG. 5(h)

```
                 701                                                  750
MMP19            RYY...VLAR GGLQVEPYYP RSLQD.WGGI PEEVSGALPR PDGSIIFFRD
Matrilysin       .......... .......... .......... .......... ..........
Gelatinase A     KFWRYNEVKK K...MDPGFP KLIADAWNAI PDNLDAVVDL QGGGHSYFFK
Gelatinase B     RLWRFDVKAQ M...VDPRSA SEVDRMFPGV P..LDTHDVF QYREKAYFCQ
Stromelysin 1    KYWRFDEKRN S...MEPGFP KQIAEDFPGI DSKIDAVF.E EFGFFYFFTG
Stromelysin 2    KYWRFDENSQ S...MEQGFP RLIADDFPGV EPKVDAVL.Q AFGFFYFFSG
Stromelysin 3    DYWRFHPSTR R...VDSPVP RRATDWRGVP SEIDAAFQDA D.GYAYFLRG
Collegenase 1    KYWRYDEYKR S...MDPGYP KMIAHDFPGI GHKVDAVF.M KDGFFYFFHG
Collegenase 2    QFWRYDNQRQ F...MEPGYP KSISGAFPGI ESKVDAVF.Q QEHFFHVFSG
Collegenase 3    QVWRYDDTNH I...MDKDYP RLIEEDFPGI GDKVDAVY.E KNGYIYFFNG
MMP12            QYWRYDERRQ M...MDPGYP KLITKNFQGI GPKIDAVFYS KNKYYYFFQG
MMP18            GYWQWDELAR TDF...SSYP KPIKGLFTGV PNQPSAAMSW QDGRVYFFKG
mt1MMP           KYYRFNEELR A...VDSEYP KNIKVWEGIP ESPRGSFMGS DEVFTYFYKG
mt2MMP           RYWRFNEETQ R...GDPGYP KPISVWQGIP ASPKGAFLSN DAAYTYFYKG
mt3MMP           RYWRYSEEMK T...MDPGYP KPITVWKGIP ESPQGAFVHK ENGFTYFYKE
mt4MMP           LYWRYDDHTR ...HMDPGYP AQ.SPLWRGV PSTLDDAMRW SDGASYFFRG

Consensus        KYWRYDE--R ----MDPGYP K-I---FPGI PSKVDAV--- --GF-YFF-G


                 751                                                  800
MMP19            DRYWRLDQAK LQATTSGRWA TELPWMGCWH .......... ..ANSGSALF
Matrilysin       .......... .......... .......... .......... ..........
Gelatinase A     GAYY.LKLEN QS.LKSV.KF ...GSIKSDW LGC*...... ..........
Gelatinase B     DRFY.WRVSS RSELNQVDQV ...GYVTYDI LQCPED*... ..........
Stromelysin 1    SSQLEFDPNA KKVTHTLKSN ...SWLNC*. .......... ..........
Stromelysin 2    SSQFEFDPNA RMVTHILKSN ...SWLHC*. .......... ..........
Stromelysin 3    RLYWKFDPVK VKALEGFPRL VGPDFFGCAE PANTFL*... ..........
Collegenase 1    TRQYKFDPKT KRILTLQKAN ...SWFNCRK N*........ ..........
Collegenase 2    PRYYAFDLIA QRVTRVARGN ...KWLNCRY G*........ ..........
Collegenase 3    PIQFEYSIWS NRIVRVMPAN ...SILWC*. .......... ..........
MMP12            SNQFEYDFLL QRITKTLKSN ...SWFGC*. .......... ..........
MMP18            KVYWRLNQ.Q LRVEKGYPRN ISHNWMHCRP RTIDTTPSGG NTTPSGTGIT
mt1MMP           NKYWKFNNQK LKVEPGYPKS ALRDWMGC.. .......... ..........
mt2MMP           TKYWKFDNER LRMEPGYPKS ILRDFMGCQE HVEPGPRWPD VARPPFNPHG
mt3MMP           GVLEIQTTRY SRLEPGHPRS ILKDLSGCD. .......... ..........
mt4MMP           QEYWKVLDGE LEVAPGYPQS TARDWLVCGD SQADGSVAAG VDAAEGPRAP

Consensus        --YWKFD--- LRVT-G-P-N ----WLGC-- ---------- ----------
```

FIG. 5(i)

```
                801                                                      850
MMP19           *..... ..... ..... ..... .....
Matrilysin      ..... ..... ..... ..... .....
Gelatinase A    ..... ..... ..... ..... .....
Gelatinase B    ..... ..... ..... ..... .....
Stromelysin 1   ..... ..... ..... ..... .....
Stromelysin 2   ..... ..... ..... ..... .....
Stromelysin 3   ..... ..... ..... ..... .....
Collegenase 1   ..... ..... ..... ..... .....
Collegenase 2   ..... ..... ..... ..... .....
Collegenase 3   ..... ..... ..... ..... .....
MMP12           ..... ..... ..... ..... .....
MMP18           LDTTLSATET TFEY*..... ..... ..... .....
mt1MMP          ...PSGGRPD EGTEEETEVI IIEVDEEGGG ..... .AVSAAAVVL
mt2MMP          GAEPGADSAE GDVGDGDGDF GAGVNKDGGS RVVVQMEEVA RTVNVVMVLV
mt3MMP          ..GPTDRVKE GHSPPDDVDI VIKLDNTAS. ..... .TVKAIAIVI
mt4MMP          PGQHDQSRSE DGYEVCSCTS GASSPPGAPG PLVAATMLLL LPPLSPGALW

Consensus       ---------- ---------- ---------- ---------- ----------


                851                                          889
MMP19           ..... ..... ..... .....
Matrilysin      ..... ..... ..... .....
Gelatinase A    ..... ..... ..... .....
Gelatinase B    ..... ..... ..... .....
Stromelysin 1   ..... ..... ..... .....
Stromelysin 2   ..... ..... ..... .....
Stromelysin 3   ..... ..... ..... .....
Collegenase 1   ..... ..... ..... .....
Collegenase 2   ..... ..... ..... .....
Collegenase 3   ..... ..... ..... .....
MMP12           ..... ..... ..... .....
MMP18           ..... ..... ..... .....
mt1MMP          PVLLLLLVLA VGLAVFFFRR HGTPRRLLYC QRSLLDKV*
mt2MMP          PLLLLLCVLG LTYALVQMQR KGAPRVLLYC KRSLQEWV*
mt3MMP          PCILALCLLV LVYTVFQFKR KGTPRHILYC KRSMQEWV*
mt4MMP          TAAQALTL*. ..... ..... .....

Consensus       ---------- ---------- ---------- ---------
```

FIG. 6

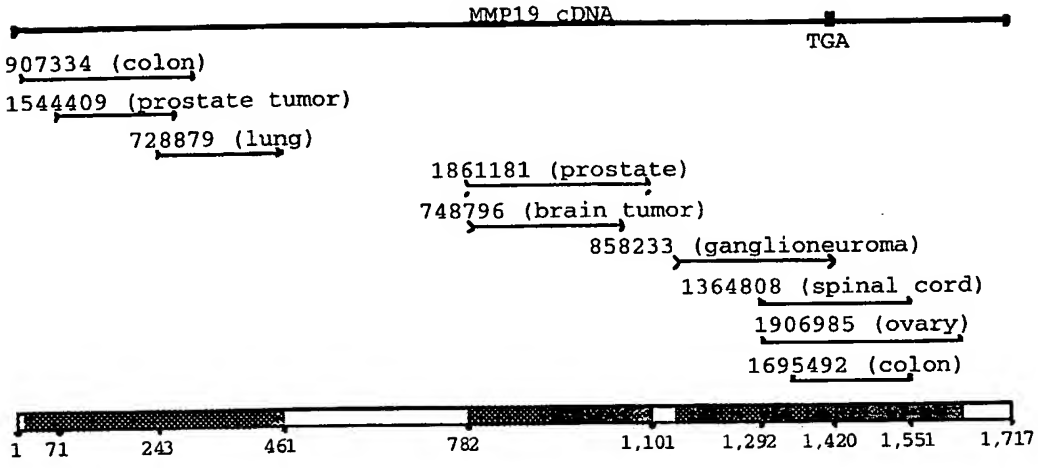
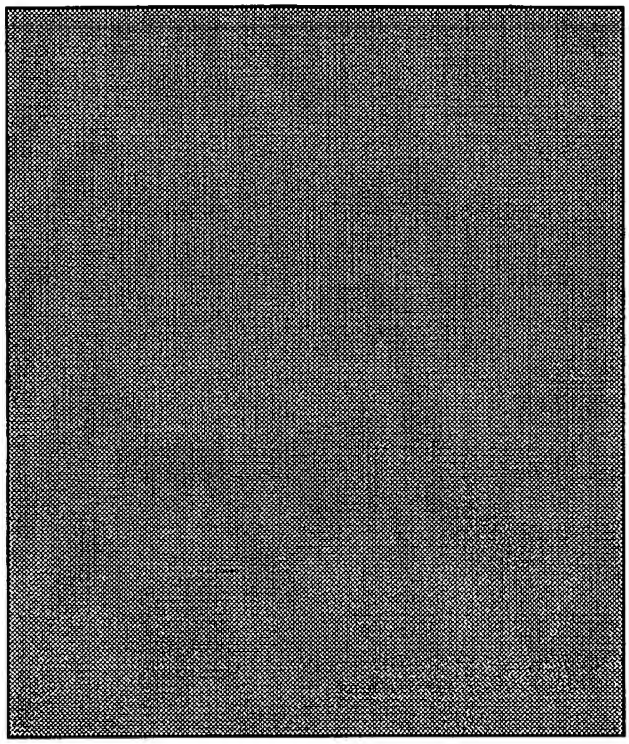

MMP19 cDNA
TGA
907334 (colon)
1544409 (prostate tumor)
728879 (lung)
1861181 (prostate)
748796 (brain tumor)
858233 (ganglioneuroma)
1364808 (spinal cord)
1906985 (ovary)
1695492 (colon)
1
71
243
461
782
1,101
1,292
1,420
1,551
1,717

FIG. 7



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/04694

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N9/64

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A.M. PENDAS ET AL., : "Identification and characterization of a novel human matrix metalloproteinase with unique structural characteristics, chromosomal location, and tissue distribution" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 7, 14 February 1997, BETHESDA, MD, US, pages 4281-4286, XP002040910 see the whole document and specially figure 1 ---	1-3,5-8, 12,14, 15,20,24
X	R.SEDLACEK ET AL., : "RASI-1, a novel autoantigen in rheumatoid arthritis" IMMUNOBIOLOGY, vol. 194, no. 1-3, 1995, STUTTGART,DE, page 153 XP002070936 see the whole document ---	1-3,5-8, 12,14, 15,20,24

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 July 1998	21/07/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mateo Rosell, A.M.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/04694

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	J. A. COSSINS ET AL., : "Identification of MMP-18, a putative novel human matrix metalloproteinase" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 228, 1996, pages 494-498, XP002036483 see the whole document	1-3,5-8, 12,14, 15,20,24
A	WO 92 09701 A (INST NAT SANTE RECH MED) 11 June 1992 see the whole document	1-12, 18-23
A	EP 0 522 169 A (FUJI YAKUHIN KOGYO KK) 13 January 1993 see the whole document	22,23
A	Q.A. SANG AND D.A. DOUGLAS: "Computational sequence analysis of matrix metalloproteinases" JOURNAL OF PROTEIN CHEMISTRY, vol. 15, no. 2, 1996, pages 137-160, XP000197027 see the whole document	1-11
P,X	C. KOLB ET AL., : "The matrix metalloproteinase RASI-1 is expressed in synovial blood vessels of a rheumatoid arthritis patient" IMMUNOLOGY LETTERS, vol. 57, 1997, AMSTERDAM, NL, pages 83-88, XP002070938 see the whole document	1-3,5-8, 12,14, 15,20-22
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P,X	WO 97 40157 A (TAKEDA CHEMICAL INDUSTRIES LTD) 30 October 1997 see the whole document and specially page 88	1-3,5-9, 12-18, 20,23,24
P,X	WO 97 19178 A (UNIV KONSTANZ) 29 May 1997 see the whole document and specially SEQ.ID.N.1	1-3,5-9, 12-17,24

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Im. tional Application No
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